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ANNUAL REPORT

MENTAL HEALTH INTRAMURAL RESEARCH PROGRAM

Division of Clinical and Behavioral Research
Division of Biological and Biochemical Research
Division of Special Mental Health Research
NATIONAL INSTITUTE OF MENTAL HEALTH

October 1, 1977 - September 30, 1978

VOLUME II - INDIVIDUAL PROJECT REPORTS

ANNUAL REPORT of program activities

MENTAL HEALTH INTRAMURAL RESEARCH PROGRAM -
Division of Clinical and Behavioral Research,
Division of Biological and Biochemical Research, and
Division of Special Mental Health Research

U.S. NATIONAL INSTITUTE OF MENTAL HEALTH

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00092-04 BP

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Central Amines and Psychiatric Illness

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SUMMARY OF WORK (200 words or less - underline keywords)

A four-year collaboration between the National Institute of Mental Health (NIMH) and the National Naval Medical Center (NNMC) has resulted in the study of central amine metabolites in the cerebrospinal fluid (CSF) of about 140 psychiatric patients, largely personality disorders and alcoholics. Results to date indicate that aggressive behavior may be inversely correlated with CSF 5-hydroxindoleacetic acid (5HIAA) and positively correlated with CSF 3-methoxy-4-hydroxyphenylglycol (MHPG). Alcoholics appear to have an increased 5HIAA in relation to acute intoxication. Disulfiram (Antabuse) appears to lower CSF homovanillic acid (HVA) and appears to increase serum norepinephrine (NE) Within these populations neither clinical depression nor aggressive behavior appears to be related to alcoholism. Data is being analyzed for subsequent publications. These patients have also been used as control data for other psychiatric diagnostic groups at NIMH.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: A growing body of evidence obtained in recent years indicates that epinephrine (E), norepinephrine (NE), dopamine (DA), serotonin (5HT), and gamma-amino butyric acid (GABA) act as central nervous system neurotransmitters and/or modulators of central nervous system transmission. Although considerable indirect pharmacologic evidence has linked functional abnormalities in these amine systems with psychiatric illnesses (particularly) affective illness and schizophrenia), the relative lack of direct data in man has limited the applicability of these hypotheses to improved diagnosis and treatment of the major psychiatric disorders. Relatively little direct data from man can be immensely valuable in making use of the massive data from animals and assessing the differences and similarities between man and animals. As well, recent data suggests that affective disorders and psychoses may yield to biological sub-typing which would enable a more effective choice of pharmacological therapy for individual patients. In addition, there is virtually no data on central neurochemical function in the various characterological disorders - a rather striking deficit in our knowledge, considering the evidence suggesting that some character disorders have patterns of family history transmission suggestive of a genetic component. Recent evidence from animals suggesting a close correlation between aggressive behavior and biogenic amines, for example, renders this a particularly interesting area for exploration at this time. Alcoholism is also an area in which neurotransmission needs more study. The purpose of this present project would be to extend the studies of central amine turnover into larger populations of psychiatric patients including manic-depressive psychosis, schizophrenia, various personality disorders, and alcoholism.

Methods Employed: Recently at the NIMH a technique has been developed which allows for the direct estimation of brain amine turnover in psychiatric patients. This method involves the administration of probenecid, a drug which blocks the process by which the amine metabolites are removed from the brain-cerebrospinal fluid (CSF) system into the blood. Following blockade of their removal, the metabolites of the amines accumulate in the spinal fluid at a rate which is in direct proportion to their synthesis or turnover rate in the brain. Experiments with this technique conducted over the last seven years at the Clinical Center of the NIH have led to the following conclusions: (1) experience in over 400 patients has indicated that both the lumbar puncture and the administration of probenecid are safe procedures not associated with significant side effects or toxicity, except for nausea; (2) studies of this technique in conjunction with specific amine precursors and synthesis inhibitors have provided direct evidence that the probenecid induced accumulation of amine metabolites does reflect amine synthesis and turnover rates; (3) application of this technique has been shown to be capable of uncovering differences in central amine function among subgroups of patients with manic-depressive illness and other functional psychotic states. These differences were often not detectable by measurement of amine metabolite baseline levels without probenecid.

Major Findings: About 140 patients have participated in this study to date. Preliminary findings suggest that a group of personality disorders with problems secondary to poor impulse control, high levels of anger-hostility, and poor judgment, when compared to a mixed control group from NIMH (normals) and patients with non-CNS neurological disorders), have similar baseline 5-hydroxyindoleacetic acid (5HIAA), homovanillic acid (HVA), and 3-methoxy-4-hydroxyphenylglycol (MHPG); however a small group (N=8) shows a significantly elevated vanillylmandelic acid (VMA). Furthermore, results to date indicate that aggressive behavior may be inversely correlated with 5HIAA and positively correlated with MHPG. Data is being analyzed for subsequent publications. A group of personality disorders have also shown no statisically significant difference in CSF cyclic 3, 5-adenosine monophosphate (c-AMP) from neurological patients with non-CNS disorders or from groups of depressive, manic, and schizophrenic patients, though aggressive behavior is positively correlated with c-AMP. VMA, dopamine beta-hydroxylase (DBH), GABA, and NE CSF assays have been done on patients, though evaluations of the data are still in process.

A group of alcoholics, now about 110, have been studied utilizing the assays above. It has been found that alcoholics do not differ from personality disorders with respect to HVA. However, early studies do indicate that the mean 5HIAA is higher in the intoxication-withdrawal stage and decreases over time in relation to the length of abstinence to reach a baseline value not differing from the personality disorders. An additional study shows that though HVA values do not change in relationship to the time period post intoxication-withdrawal, they are depressed concomitant with the use of disulfiram (Antabuse), a DBH inhibitor. In addition, disulfiram use correlates with a significant increase in serum NE. Mean serum DBH in alcoholics vs. normal controls was significantly lower, blood pressure was significantly higher, and serum NE was not different. Other studies indicate that lower DBH is correlated with increasing psychopathology as measured by the Minnesota Multiphasic Personality Inventory (MMPI) and lower CSF DBH is associated with disulfiram induced psychoses. Furthermore, low platelet monoamine oxidose (MAO) and elevated erythrocyte catechol-methyl transferase (COMT) is associated with disulfiram induced psychoses. A number of studies involving MAO in platelets and COMT in erythrocytes of alcoholics are also underway.

Other studies show that neither clinical depression nor aggressive behavior in this group of early to mid-stage alcoholics can be associated with alcoholism. Aggressive behavior can be associated with alcoholism when the alcoholics are also compulsive personality disorders.

Significance for Mental Health Research: Central cerebral functioning is greatly under-studied in two major groups of psychiatric patients, namely personality disorders and alcoholics. Studies of animal models and hyperactive children suggest a relationship between central neurotransmitter systems and aggressive behavior. The neurobiological aspects of alcoholism, either predisposing, concomitant, or resultant, are of timely significance as alcoholism is a prevalent problem. Also drug-free personality disorders serve as a useful comparison group for biological studies of other psychiatric disorders.

Proposed Course of Project: The preparation for this project began in January 1973. The approval processes, both in terms of scientific merit and the protection of rights of patients, were completed in July 1974. The firs lumbar puncture was performed in September 1974. This project was submitted to the Navy for reapproval in March 1977. We believe this collaboration continues to be of mutual benefit to NIMH and NNMC.

Publications:

Smith CC, Tallman JF, Post RM, van Kammen DP, Jimerson DC, Brown GL, Brooks BR, Bunney WE Jr: An examination of baseline and drug-induced levels of cyclic nucleotides in the cerebrospinal fluid of control and psychiatric patients. Life Sci 19:131-136, 1976.

Major LF, Zeigler MG, Lake CR, Brown GL: The effects of disulfiram on plasma norepinephrine in male alcoholics, in Currents in Alcoholism, Vol 1. Edited by Seixas FA. New York, Grune & Stratton, 1977 pp 197-209.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00021-13 BP

PERIOD COVERED

October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Studies of Sleep

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☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Experimental findings over the past decade promise understanding of the basic biochemical process underlying sleep and the increasingly clear demonstrations of severe sleep disturbances associated with psychiatric disorders, as well as the existence of sleep disorders themselves, indicating need for better means of alleviation. To the extent that the process of sleep can be understood, more effective treatment of disorders of sleep will surely follow. In addition to the clinical, biochemical and endocrinological work this laboratory has done in the past, we are beginning to ask fundamental questions about the nature of sleep, using chemical and psychological probes.

These studies contribute to the extensive national and international efforts to understand why we spend one-third of our lives in this state.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives:

Experimental findings over the past decade promise understanding of the basic biochemical processes underlying sleep and increasingly clear demonstrations of the severity of sleep disturbances in many psychiatric patients indicate the need for better means of alleviation. To whatever extent understanding of sleep can be achieved, more effective treatment of its disturbance will surely follow.

Major Findings: Pharmacological

I. Cholinergic

A. Relationship of Cholinergic Activity to REM Narcoleptic Attacks

In order to explore the possible relationship between levels of cholinergic activity and REM narcoleptic attacks, we have begun therapeutic trials of the cholinergic blocking agent, Artane (trihexyphenidyl 2 mg tid). Results from the first three narcoleptic patients have been encouraging in that all three showed clinical improvement, especially in terms of less frequent attacks of cataplexy. The converse procedure of administering physostigmine (0.5 mg IV q 5 min. to a total dose of 1.5-2.5 mg) in an effort to determine whether increased cholinergic activity induces REM narcoleptic cataplexy did not have any discernible effects in the same three patients. In addition, physostigmine had no apparent effects on the mood of these patients, contrary to recent reports involving psychiatric patients.

B. Time-dependent Effects of Physostigmine on Normal Human Sleep and Arousal

We have recently found that REM sleep was induced by 0.5 mg physostigmine (phy) i.v. 35 min after sleep onset, in normal subjects pretreated with methscopolamine (0.5 mg IM). We have now extended the study to include other time periods. Thirty-three normal volunteers received 40 physostigmine (phy) and 40 matched saline (sal) infusions. (1) With postinfusion-arousals excluded, infusions given 5 mins after sleep onset reduced time from infusion to onset of REM from 78.9 $\pm$ 14 min (sal) to 52.4 $\pm$ 10.4 (phy) ($p<0.05$, two-tailed "t" test). (2) Infusions given 35 min after sleep onset reduced time from infusion to onset of REM from 65.7 $\pm$ 15.2 (sal) to 11.2 $\pm$ 2.1 (phy) ($p<0.005$). REM was induced more rapidly by physostigmine infusions given 35 min after than 5 min after sleep onset ($p<0.01$). (3) For the infusions at REM onset, 5 min after and 25 min after REM, the predominant effect was one of arousal. Judging from number and duration of awakenings, the order of intensity of arousal was: REM > 5 min after > 25 min after REM. Apart from "true" awakenings, two other types of arousals were seen:

Also occurring EEG/EMG dissociation with alpha EEG, muscle atonia and rapid eye movements resembling sleep paralysis. This was seen in three subjects who were given 1.0 mg dose and was accompanied by early morning reports resembling sleep paralysis (e.g., "I was awake but couldn't move my arms."), "alpha REM" characterized by periods of alpha EEG with muscle atonia and without eye movements alternating with conventional REM sleep.

These data suggest that cholinergic mechanisms are involved in initiating REM sleep and arousal. During the first REM period, physostigmine more readily induces REM when given 35 min than when given 5 min after sleep onset, whereas the same dose given at REM onset and during the second NREM period produces different intensities of arousal.

Using single bolus infusions of physostigmine administered at different times during the first and second NREM periods, we have recently shown that REM sleep and arousal can be induced in a time- and dose-dependent manner (1). In view of the extremely short-lived action of a bolus infusion of physostigmine we have hitherto not been able to determine if the duration of a REM period can be prolonged in addition to triggering its onset.

Seven normal male volunteers (Mean age 22.8) were studied for three nights (one "adjustment" and two experimental nights). Subjects were pretreated with 0.75 mg IM methscopolamine prior to bedtime; and during sleep they received either placebo or physostigmine 1.0 mg given by a slow IV drip over a period of 60 minutes. The IV infusion began 35 minutes after sleep onset and ended about 95 minutes after sleep onset.

Physostigmine significantly decreased both the latency of the first REM period (Placebo = 108.4 $\pm$ 16.2 min (mean $\pm$ S.E.) physo = 61.7 $\pm$ 2.2 min, $p<.05$ and the duration of the second NREM period (placebo - 110.4 $\pm$ 13.6, physo - 74.4 $\pm$ 6.5, $p<.05$). The duration of individual REM periods were not altered. Although total REM time and REM per cent showed a non-significant increase of physostigmine (RT = 98.2 $\pm$ 6.4 min, R% = 25 $\pm$ 1 5%) as compared to placebo nights (RT - 80.9 $\pm$ 9.4, R% = 20.1 $\pm$ 1.9%) this can be accounted for by the greater number of REM periods on physostigmine (4.3 $\pm$ 0.3) than on placebo (3.6 $\pm$ 0.2) conditions. Cholinergic mechanisms may modulate the "timing" rather than the "maintenance" of REM.

To examine the psychological process of dreaming in man, seventeen normal volunteers (11M, 6F, mean age 23) after pretreatment with methscopolamine 0.5 mg IM slept with an IV needle and at either 10 or 35 minutes after sleep onset received one intravenous infusion per night of either placebo or physostigmine 0.5 mg.

During Experiment I subjects were given an IV infusion 10 minutes after sleep onset and were awakened 20 minutes after infusion. Experiment II includes both 7 and 20 minute awakenings; in addition subjects were instructed to go back to sleep after the 20 minutes awakening and were then awakened approximately 7 minutes after they had spontaneously gone into REM sleep. The above study was designed to control for (1) time of infusion, (2) time from infusion to awakening, (3) state of sleep during awakening.

At each awakening a taped interview was conducted. Subjects were questioned waking. After the interview subjects filled out a questionnaire, a dream mentation scale was constructed from the questionnaire ranging from +5 (absolute confidence in presence of dream) to -5 (absence of dreaming).

	Experiment I		Experiment II				
	Awakenings 20 min after infusion		Awakenings 7 min after infusion		Awakenings 20 min after infusion		Spontaneous REM sleep awakenings
	Physo	Placebo	Physo	Placebo	Physo	Placebo	
1) No. of awakenings	10	10	9	9	10	10	10
2) Sleep state during awakenings	non-REM	non-REM	non-REM	non-REM	REM (9) non-REM(1)	non-REM	REM
3) No. positive dream recall (%)	4(40%)	2(20%)	4(44%)	3(33%)	8(88%)*	2(22%)	7(70%)
4) Dream mentation (mean ± SEM)	not obtained		-0.4	-1.2 ±1.3	+2.3* ±0.9	-2.0 ±0.8	+2.3 ±0.9

* $p<.01$

During Experiment I and the 7 minute post-infusion awakenings of Experiment II neither REM sleep nor dreaming was induced by physostigmine. In the 20 minute post-infusion, physostigmine induced the onset of REM sleep in approximately 13 minutes in all but one subject. Those drug-induced REM episodes were associated with significantly higher incidence of dream recall than placebo.

This study indicates that the physostigmine-induced physiological characteristics of REM sleep are accompanied by dreaming. The dreaming occurring during physostigmine-induced REM periods appears to be similar to that observed during spontaneously occurring REM sleep.

Induction of REM Sleep in Man by Arecholine, a Cholinergic Muscarinic Agonist. Blockade by Scopolamine.

Ten normal volunteers (8M, 2F, mean age 25.3) were each studied on 4 randomly ordered experimental nights (after a night of adaptation). After pretreatment with methscopolamine 0.5 IM on three of the experimental nights, they each received one intravenous infusion about 32 to 35 min after sleep onset of either (1) placebo, (2) arecholine 1.0 mg IV, or (3) arecholine 1.5 mg IV. On the fourth night pretreatment was scopolamine 0.5 mg IM followed by IV infusion of arecholine 1.5 mg. The results were: (1) Both arecholine 1.0 mg and 1.5 mg triggered the onset of REM sleep within 20.1 and 15.9 min after infusion respectively. (2) Pretreatment with scopolamine blocked the REM-inducing effect of arecholine 1.5 mg IV. (3) Once the onset of the first REM period was moved forward subsequent nonREM-REM cycles were advanced without altering REM-REM intervals. (4) The number of REM periods was significantly increased after arecholine 1.0 mg (4.1±0.3) and 1.5 mg (4.5±0.2) compared to placebo (3.5±0.2). (5) Total REM time and percent after arecholine 1.5 mg (100.7±6.4 min, 24.7±1.7%) was significantly greater than placebo (76.2±5.4 min, 18.1±0.9%). The data with arecholine, a specific cholinergic muscarinic agonist, confirms our previous findings with physostigmine and suggests that acetylcholine may play a role in the triggering of REM sleep in man.

Experimental Shortening of REM Sleep Ultradian Rhythm with Timed Multiple Infusions of Arecholine.

Seven normal volunteers (4M, 3F, mean age 25 yrs) were studied under the following two experimental conditions. (1) Pretreatment with methscopolamine 0.5 mg IM followed by three IV infusions of arecholine (1.5 mg, 1.0 mg and 1.0 mg doses) given at the following three times respectively: (a) 35 min after sleep onset; (b) 30 min after end of first REM period; (c) 30 min after end of second REM period. (2) Pretreatment with methscopolamine followed by three placebo (saline) IV infusions. Subjects were run either in pairs or groups of 3 such that the timing of the placebo infusions was "yoked" to the arecholine infusions.

TABLE: Duration (minutes) of NREM and REM periods (mean ± SEM)

	$NREM_1$	REM_1	$NREM_2$	REM_2	$NREM_3$	REM_3	$NREM_4$	REM_4	$NREM_5$	REM_5
Placebo	95.7 ±14.7	14.1 ±3.9	89.1 ±5.7	22.8 ±3.2	78.4 ±4.6	25.5 ±4.7	-	-	-	-
Arecholine	42.2* ±2.6	10.3 ±2.4	38.3* ±1.3	12.8* ±2.5	41.4* ±3.7	21.0 ±4.9	84.7 ±4.1	22.1 ±5.4 (N=6)	65.4 ±4.6 (N=6)	28.8 ±5.0

*p<.01

The results of this study are: (1) It was possible to transform a normal REM ultradian rhythm (with REM-REM interval of about 100 min) into a rhythm with REM_1-REM_2 interval of 48.6 min and REM_2-REM_3 interval of 54.2 min. (2) There were a significantly greater number of REM periods after arecholine (5±0.22) than placebo (3.71±0.29) resulting in greater REM time and percent (arecholine = 104.1±1.5.6 min, 24.8±1.9% and placebo = 84.4±4.6 min, 20.1±1.2%). (3) Duration of individual REM periods were either unchanged or shortened (e.g., REM_2). Acetylcholine plays a role in the timing of REM sleep. The increase in REM time seen appears to result from an increase in the number but not the duration of REM periods. There also appears to be a "ceiling" after which REM time cannot be further increased by cholinergic agents since both single and multiple infusions of arecholine produce the same amount of increase in REM time.

E. Acetylcholine and Sleep: Effect of Oral Choline on Normal Human Sleep.

Choline, a normal dietary constituent, has recently been shown to be a precursor of brain acetylcholine. If so, in the light of our previous work with physostigmine and arecholine (see previous abstracts: Acetylcholine and Sleep: I and II), we would predict that choline administered to humans may alter REM sleep parameters. A double blind crossover design with 12 normal male subjects was used. Subjects slept for 3 nights (1 adaptation and 2 experimental) and just prior to "lights out" (11:30 p.m.) were given an elixir containing either 10 g of choline chloride or placebo matched for taste, color and consistency. The results were: (1) Choline did not have any effect on conventional sleep parameters when data from all 12 subjects were analyzed. (2) When the subjects were rank ordered with respect to their placebo (baseline) REM latency, REM time and REM %, we found that low placebo REM latencies were increased and high placebo REM latencies were decreased by choline. The correlation between placebo (baseline) REM latency and the change in REM latency after choline (i.e., choline-placebo value) was inversely correlated $r=-0.90$, $N=12$ ($p<.01$). Similarly there was an inverse correlation between placebo REM time and REM % and change after choline ($r=0.63$ for REM time and $r=0.61$ for REM%, both $p<.05$). Whether the above changes in REM parameters produced by choline represents a genuine central neuromodulatory effect or is no more than a statistical artifact is unclear. Further experiments to test this hypothesis as well as the effect of intravenous choline chloride administered during sleep in normal volunteers is currently underway and will be discussed at the meeting.

F. The Effect of Piperidine, a Cholinergic Nicotinic Agent on Normal Human Sleep.

Piperidine is an alicyclic amine with nicotine-like cholinomimetic effects on central and peripheral nervous system. In view of conflicting reports on the effect of piperidine on REM Sleep in rodents, we undertook to study the effect of oral piperidine Hcl in normal volunteers. Using a 4x4 Latin square design, 8 subjects were recorded on 4

nights under the following four experimental conditions (a) placebo, (b) piperidine 200 mg, (c) piperidine 400 mg and (d) piperidine 600 mg. All medications were given prior to "lights out" (11:30 p.m.). No change in any sleep parameter including REM latency, REM time and REM density were observed after piperidine. Although data from animal pharmacology suggests that the doses used in our study would have central effects, the possibility still remains that physiologically effective doses were not used. Intravenous administration of piperidine during sleep is also currently underway.

REM Deprivation Increases Hippocampal Acetylcholine Turnover.

Human pharmacologic studies and animal single cell recording observations suggest that cholinergic neurons may play a role in the regulation of rapid eye movement (REM) sleep. In order to further examine this relationship, we have examined turnover of acetylcholine (ACh) and concentrations of ACh and choline in REM sleep-deprived rats and controls. REM deprivation was performed by the "flower pot" method described previously, which reduces REM sleep by 50% without affecting nonREM sleep. This involves four experimental groups: (1) rats standing on top of a 6.5 cm inverted flower pot surrounded by water (REM deprived group); (2) rats on a 12.5 cm flower pot (stress control); (3) rats who swim in 10 cm of water for one hour per day (stress control); and (4) untreated rats. After four days of these procedures, phosphoryl $(C^2H_3)_3$ choline was infused intravenously, for nine minutes. The animals were then sacrificed by microwave radiation, and the brains dissected into cortex, striatum, hippocampus, and thalamus. Determinations were performed by a gas chromatography-mass spectrometer procedure.

There were small increases in concentrations of ACh and choline in the thalamus of two types of stress control animals. More striking however, was a significant ($p<0.01$ by Dunnets t-test) increase in ACh turnover in the hippocampus of the REM-deprived rats (see Figure), which did not occur in naive or stress control rats.

This data supports the participation of cholinergic mechanisms in REM sleep regulation but fails to unveil the transsynaptic link which is operative in activating septal-hippocampal cholinergic neurons. Several lines of independent investigations suggest that septal-hippocampal cholinergic neurons are activated transsynaptically by noradrenergic and peptidergic axon terminals, which may have been affected by REM deprivation.

Identification of a Stimulatory Cholinergic Mechanism in Sleep-related Growth Hormone Secretion.

We have examined the effects of cholinergic blockade with methscopolamine bromide, 0.5 mg intramuscularly, on sleep-related and insulin-induced growth hormone (GH) and prolactin (PRL) secretion. Thirteen normal young men were studied; eight had sleep studies, and at different

times in 3 sleep subjects and in 5 others not studied during sleep, insulin tolerance tests were performed with 0.1 unit/kg insulin. After an adjustment night in the sleep laboratory, saline control and methscopolamine studies were done in a random sequence; study procedures included electroencephalographic, electromyographic, and electro-oculographic recordings, and blood sampling every 20 minutes for hormone radio-immunoassays. On methscopolamine nights, the mean $\pm$ SEM overall GH level of 2.89 $\pm$ 0.44 and the mean peak GH level of 11.09 $\pm$ 3.11 ng/ml were dramatically reduced to 0.75 $\pm$ 0.04 and 1.04 $\pm$ 0.25 ng/ml, respectively ($p<0.0001$ and $p<0.001$). Despite virtual absence of GH secretion during the night in every study subject, no measured sleep characteristic was significantly affected by methscopolamine, including percentage slow-wave sleep (12.1 $\pm$ 2.6% control vs. 10.3 $\pm$ 2.5% drug). Sleep PRL concentrations were not changed by methscopolamine. In contrast to the abolition of sleep-related GH secretion, administration of methscopolamine only marginally reduced the GH response to insulin hypoglycemia. Only one of ten time points differed significantly, and the peak concentrations, mean increments, and areas under the curves were not significantly different. We conclude that the burst of GH secretion which normally occurs following sleep onset is primed by a cholinergic mechanism which does not influence slow-wave sleep. Cholinergic mechanisms outside the central nervous system do not appear to play an important role in sleep-related prolactin secretion. The neurotransmitter mechanisms, and presumably the pathways, which subserve sleep-related GH secretion in man appear to be different from those which mediate the GH response to pharmacologic stimuli such as insulin.

I. Selective Enhancement of Serial Learning and Encoding of Low Imagery Words in Man.

The effect of a single oral dose of choline chloride (10g) on two kinds of memory measures (serial learning and a selective reminding task) was studied in 10 normal volunteers. Choline decreased significantly the number of trials required to master a serial learning word test. On the selective reminding task only the recall of poorly imageable words (which are ordinarily difficult to remember) was enhanced by choline. Words which were highly imageable (and ordinarily easy to remember) were unaffected.

The degree of enhancement with choline correlated inversely with performance of subjects on placebo. The cholinergic agonist, arecholine (4mg), significantly enhanced serial learning in normal human subjects. Subjects were pretreated with methscopolamine prior to both arecholine and placebo injections. Conversely, scopolamine (0.5mg), a cholinergic antagonist, impaired learning and this impairment was reversed by a subsequent injection of arecholine. The degree of enhancement produced by arecholine and the impairment after scopolamine were inversely proportional to subject's performance on placebo; i.e., "poor" performers were more vulnerable to

both the enhancing effect of cholinergic agonist and precursor and the impairment after cholinergic antagonist then "good" performers. Our data indicate that brain acetylcholine may play a facilitory role in encoding and storage of specific information items in man.

I. Histamine: Effects of L-Histidine on Sleep.

There is considerable indirect evidence suggesting that histamine may function in the brain as a neurohumor in sleep-waking mechanisms. To test the possible role of histamine in the control of waking in man, we administered its amino acid precursor, L-histidine, to human subjects and evaluated their electrographic sleep patterns. L-histidine was given to three patients with intractable narcolepsy (20 gm per day for 2 weeks), to four normal volunteers (32.4 gm per day for 5 days) and to a patient with progressive systemic sclerosis (48.6 gm per day) for 16 days. No effects were observed on nocturnal EEG sleep patterns in any of the subjects or on the symptoms of the narcoleptic patients. Although the degree to which histamine levels in the brain were elevated in this study is not clear, these results do not encourage the hypothesis that histamine is an alerting or waking factor in the brain.

II. Catecholamines: Differential Effects of D- and L-Amphetamine on the Sleep of Depressed Patients On and Off Lithium Treatment.

Dextro (d-) and levo (l-) amphetamine produced different EEG sleep changes in seven depressed patients. Each patient received placebo or one of the isomers (30 mg base) at 7:25 a.m. in a double blind fashion. The patients were studied under two conditions: without treatment with lithium carbonate and with treatment with lithium carbonate (0.9-2.1 gm/day beginning a minimum of 10 days before study). Both isomers reduced REM sleep and the proportion of total sleep spent in REM (the REM%). No REM rebound was observed on the night following REM suppression. Only d-amphetamine delayed sleep onset and reduced Total sleep time, NREM sleep time, and sleep efficiency. The same changes were observed with and without lithium carbonate treatment.

V. Serotonin:

. Relationship to Narcolepsy.

Since serotonin has been implicated in the induction and maintenance of sleep, we administered para-chlorophenylalanine (PCPA 3 gm/day for up to 4 weeks) to 3 patients with narcolepsy. No consistent change in sleep or clinical symptomatology was observed in the 3 patients as a group. One patient, in contrast to our previous experience with PCPA in patients with carcinoid syndrome, actually showed a significant increase in REM while on PCPA, while the other patients showed no decrease in REM. These results suggest (a) that narcolepsy is not related to an abnormality of serotonin and (b) that narcoleptics respond differently to PCPA than carcinoid patients.

B. Reduction of Brain Serotonin and REM Sleep by Acute Administration of a Tryptophan-free Amino Acid Diet

A variety of studies have suggested that the serotonergic system plays an important role in the regulation of sleep. In order to clarify this role, we have performed sleep studies on rats in whom brain serotonin was decreased by a new technique-the acute administration of a tryptophan-free amino acid diet.

Prior to the sleep study we confirmed the efficacy of this method in a biochemical study on 24 rats. They were trained for a 20-day period to eat their entire daily food supply (an amino acid diet) in 2 hours. On the twenty-first day they were divided into two groups who received the same diet or one of similar composition but lacking tryptophan. Animals from each group were then decapitated at intervals over the next 24 hours. At the end of the first two-hour feeding, serotonin and 5-HIAA concentrations had dropped significantly. They continued to decrease until they reached a nadir eight hours after the start of feeding, at which time serotonin and 5-HIAA levels were 30 and 41 percent of control values, respectively. At 24 hours they had started to increase but were still significantly less than control values.

Sleep studies were performed on five rats in a paired-data, crossover design. As can be seen on the Table, percentage REM sleep was decreased by one-third ($p<.05$) and there was a small but signinificant increase in nonREM sleep. Total sleep time was unchanged.

Our observation that an acute reduction in brain serotonin failed to produce decreases in nonREM sleep is in agreement with the results of two studies each in rats and humans. These data seem inconsistent with the hypothesis that the serotonergic system plays an important role in the production of nonREM sleep.

	Control Diet	Tryptophan-Free Diet	Statistical Significance
Total sleep	519.6±25.7*	529.8±27.0	NS
Sleep latency	45.5±10.6*	35.8±10.6	NS
Intermittent waking (IW/TRP)	33.9± 3.0	30.8± 2.2	NS
nonREM sleep (NR/TST)	87.6± 2.4	91.4± 2.3	$p<0.05$
REM sleep (REM/TST)	12.3± 2.4	8.5± 2.2	$p<0.05$

Values represent mean number of 30 sec. epochs ± SEM.

Effects of tryptophan-free diet on brain serotonin and 5HIAA

Hours after food presentation	Control diet		Tryptophan-free diet	
	Serotonin	5HIAA	Serotonin	5HIAA
2	1.43±0.04	0.52±0.06	0.80±0.02***	0.38±0.02**
4			0.62±0.01***	0.27±0.02***
8			0.44±0.02***	0.21±0.01***
24	1.52±0.02	0.56±0.04	0.65±0.02***	0.41±0.04*

Each value represents the mean from four rats ± S.E.M., in units of μg/g brain tissue.

* $p<0.05$ by t-test when compared to mean of combined control values.
** $p<0.01$
*** $p<0.001$

These studies contribute to the present extensive national and international efforts to unravel the complex relationships of clinical disorders, biochemistry and sleep.

Ethanol:

A Dose-response Study of the Acute Effects of Ethanol on the Sleep of Rats.

Although there is no shortage of studies on the effects of ethanol on sleep of animals, there is little dose-response data available. In order to examine this relationship more carefully, we have administered increasing doses of ethanol (1.1, 1.5, 2.0, 2.5 g/kg) and equivolume saline intraperitoneally to five groups of six rats each. Injections were given five minutes before the beginning of seven-hour recordings, from 9:00 a.m. to 4:00 p.m. The percent of REM decreased and the percent of slow-wave sleep increased. By dividing the recording into sequential 3.5 hour periods, it became clear that this effect occurred most strongly in the first 3.5 hours of sleep. Although the number of awakenings was not changed over the recording as a whole, they were significantly increased in the second half of the recording. This may be a reflection of greatly decreased blood alcohol levels. (A dose of 2.5 g/kg is completely metabolized in about six hours in the rat.) It is of interest that even the highest doses of ethanol did not produce prolongation of total sleep time in either the whole recording or the first or second halves.

Hypnotics and Ethanol: Residual EEG Effects.

The literature is divided between those who have found a possible synergistic interaction of ethanol and benzodiazepines and those who have not. We have examined this relationship by recording 12-minute waking clinical EEG's in 47 normal young men who received 0.25 mg triazolam, 30 mg flurazepam or placebo, alone or in combination with 0.8 g/kg ethanol. EEG's were performed as a baseline (3:00 p.m.), 50 minutes after receiving drug

or drug placebo (5:50 p.m.), 50 minutes after receiving ethanol or ethanol placebo (7:05 p.m.) and 16 hours after having received the drug (9:00 a.m.). Criteria for the presence of drug effects were:

1. Increase in amount and amplitude of sinusoidal rhythmic beta activity.
2. Lower amplitude of background activity.
3. Decrease in amount of alpha activity
4. Presence of "well-modulated" alpha.
5. Presence of "drug spindles."

Eighty-five percent of the baseline records were correctly identified as having no drug effect. Analysis of the second EEG (50 minutes after drug) and third EEG (50 minutes after ethanol) showed that our interpretations had a significant relationship to whether or not the subject had received the active drug ($p<0.02$ and $p<0.05$ respectively). These findings lead us to believe that this method for determining the presence of drug effects is valid.

Analysis of the fourth EEG--16 hours after drug administration--showed no overall difference in incidence of EEG drug effects among the various combinations of drugs and ethanol. Neither drug alone was different from placebo, nor was either drug alone different from itself plus ethanol.

Flurazepam plus alcohol produced drug effects in 86% of the records, compared to 22% in the placebo group ($p<0.019$; Fisher Exact Probability Test). The difference between incidence of drug effects following triazolam plus ethanol (56%) and placebo (22%) did not reach statistical significance. There was no difference in frequency of effects between flurazepam plus ethanol and triazolam plus ethanol.

These data lead us to believe that ethanol produces a small but significant increase in the duration of EEG effects of flurazepam. Flurazepam alone is known to remain active for some time (the major metabolite may have a half-life of 50-100 hours). Thus, repeated use of the drug might produce cumulative effects which may possibly be increased by ethanol.

C. Sleep, Behavior, and Blood Ethanol Concentration in the Ethanol-Dependent Rat.

In this study sleep recordings were performed to further characterize a previously-described animal model of ethanol dependence. In summary, this consists of administering approximately 10 gm/kg of ethanol per day by intragastric intubation in six divided doses for four days. Eight-hour EEG recordings were made during the baseline period and the first and third days of ethanol administration (induction period). One group of animals (N=14) was also recorded on the first and fourth withdrawal days; a second group (N=5) was recorded on days 1-4 and 6 of withdrawal. During the induction period, REM sleep time was decreased, particularly on the third ethanol day when blood ethanol concentrations were highest.

This largely reflected decreases in the number of REM sleep episodes. The first day of ethanol withdrawal was associated with a decrease in total sleep time, primarily due to loss of nonREM sleep. Total sleep time gradually returned to normal, and in fact slightly exceeded baseline levels on the third withdrawal day. At this time, REM percentage increased to twice the baseline values. This was related to a decrease in REM latency and an increase in the number of REM sleep episodes. REM percentage then decreased such that on the fourth withdrawal day it approached baseline values. Total sleep time and other sleep parameters were also undistinguishable from baseline on the fourth day. Blood ethanol concentrations on the first withdrawal day were inversely related to behavioral scores of withdrawal and to EEG sleep latency.

The major observations in this model seem consistent with observations in the literature on ethanol withdrawal in animals and humans. Several of the EEG parameters--notably the initial decreased sleep and later REM rebound--may be useful in testing new treatments for ethanol dependence.

Enzymes:

The Relation of Platelet MAO to Human Sleep.

Platelet monoamine oxidase (MAO) activity has been related to likelihood of psychiatric contact, scores on personality tests, family history of suicide, and other measures. Most studies have shown decreased activity in chronic schizophrenics and bipolar depressed patients. In the present study, we have examined the sleep of normal volunteers with high and low platelet MAO activity.

These volunteers were drawn from groups reported elsewhere, obtained by selecting individuals with MAO activity in the highest and lowest decile of a population of 375 students. Ten high [mean 16.1±3.2 (sd) nanomoles per 10^8 platelets per hour] and ten low [7.1±2.3 (sd)] volunteers, 5 men and 5 women in each group returned for sleep studies. Each subject was recorded for two nights following two adjustment nights.

The group with high platelet MAO activity had significantly lower REM sleep time (69.9±5.2 vs 92.7±8.6 minutes; $p<0.05$), lower REM density 1.5±0.1 vs 1.9±0.1, and presumably as a result of the previous two factors, a lower REM index, a measure of the total number of eye movements (105.5± 9.3 vs 184.8±21.8; $p<0.01$). All other sleep variables were similar for the two groups.

An examination of the REM sleep parameters in which the two groups differed suggests that the values for the low platelet MAO group are similar to those of other volunteers we have studied. An implication, then,

is that subjects with high platelet MAO may have less REM time and a lower REM density than might be expected in a general population. These observations may also constitute the first report relating individual differences in an enzyme involved in neurotransmitter metabolism to human sleep EEG parameters.

VII. Hormones:

A. Regulation of Sleep-Related Growth Hormone and Prolactin Secretion.

The study of pharmacologic manipulation of sleep-related growth hormone (GH) and prolactin (PRL) secretion has provided data on the roles of the serotonergic, noradrenergic and cholinergic systems in anterior pituitary regulation. The authors will review work in this area and report on two studies from their laboratory. In the first, the serotonin receptor blocker methysergide (2 mg PO q 6 h) was administered for 48 hours to normal young adults, and blood samples were drawn every 20 minutes during eight-hour sleep recordings. Methysergide administration was associated with an increase in several measures of sleep-related GH secretion. Mean peak concentrations of GH rose from 7.86±1.35 (SEM) ng/ml on control nights to 10.30±1.39 ng/ml with methysergide ($p<0.03$). Mean nocturnal PRL concentrations were decreased by 70.3%. In contrast to the increases in sleep-related GH secretion, daytime insulin-stimulated GH secretion was decreased 36.4% by methysergide.

In the second study, a single IM injection of the anticholinergic agent methscopolamine (0.5 mg IM) was found to profoundly suppress sleep-related GH secretion, reducing the mean peak concentration from 11.09± 3.11 ng/ml on control nights to 1.04±0.25 ng/ml on drug nights ($p<0.001$). Despite this virtual absence of GH secretion, no measured sleep EEG parameter was affected by methscopolamine. Sleep-related PRL secretion was also unaffected by methscopolamine. In the daytime, insulin-induced GH secretion was marginally reduced by the drug.

These studies would seem to imply that: (1) serotonergic mechanisms may have an inhibitory role, and cholinergic mechanisms may have a facilitory role, in regulation of sleep-related GH secretion; and (2) data drawn from pharmacologic manipulation of daytime insulin provocative testing may lead to erroneous conclusions regarding physiologic control of GH secretion.

B. The Effect of Dexamethasone on Sleep-Related Growth Hormone Secretion in Congenital Adrenal Hyperplasia: Therapeutic Implications.

Treatment of children with 21-hydroxylase deficiency congenital adrenal hyperplasia (CAH) with steroids has two goals: replacement of inadequate secretion of cortisol (and sometimes aldosterone) and suppression of oversecretion of adrenal androgens. In adults, and adolescents who have achieved full growth, it is most desirable to use long-acting

steroids such as dexamethasone (DEX), given at midnight. The effects of DEX on nocturnal GH secretion needs to be evaluated, however, particularly before use in children and adolescents who have not achieved full stature. For this reason we have examined GH secretion and other endocrine measures during sleep in CAH patients receiving DEX in two different regimens.

The four females and two males who participated in the study ranged in age from 13 to 22. All but two (who were female) were salt losers. All patients received 0.5 mg DEX for six days each at 0700 and 2300 hours. Sleep studies were performed on night 5, and during a study in which several blood samples were drawn on night 6.

Examination of 24 hour excretion of 17 ketosteroids and pregenanetriol showed adequate suppression under both regimens in four of the six patients. In two subjects there was inadequate suppression with either regimen. LH was found to be secreted episodically throughout the night in a similar and normal manner with both regimens. Mean nocturnal FSH concentrations were also similar.

Examination of sleep-related GH revealed increased secretion during the first two hours of sleep when DEX was given at 0700 compared with administration at 2300. The areas under the curves of GH plots during this time were 1737±744 (SEM) and 666±359 ng/ml-min respectively ($p<0.05$). Following administration at 2300, there was a tendency for increased GH secretion later at night, which did not, however, reach significance.

Only two significant differences were noted on sleep EEG recordings made during the blood study: Following 0700 administration, there was a longer sleep latency (65.8±18.1 vs 24.2±7.9 minutes; $p<0.02$) and decreased nonREM sleep (266.8±34.4 vs 363.3±13.7 minutes, $p<0.02$). These observations were not borne out by the recording on night 5, and hence are of questionable biological importance. The possibility remains, of course, that DEX affects sleep differently when patients undergo the slight stress of an intravenous study compared to when they do not.

These observations suggest that administration of DEX at 0700 suppresses the adrenals yet permits sleep-related secretion of GH. In contrast, administration at 2300 suppresses sleep-related secretion of GH. Further studies of chronic use, and considerations of possible effects on somatomedins, will be necessary to further establish the safe use of DEX in children and adolescents.

LH-RH Administration and Human Sleep.

Luteinizing hormone-releasing hormone (LH-RH), a decapeptide of hypothalamic origin, stimulates release of LH and FSH from the pituitary; in addition it may possess direct actions on the central nervous system. It has been shown, for instance, to cause mating behavior in estrogen-primed ovariectomized rats. Its administration has been reported to

produce sexual arousal in human males with hypogonadism well before androgen levels reach the normal range. Because of these implications that LH-RH may possess central effects, and since gonadal hormones have been reported to influence sleep, we have explored the possibility that LH-RH administration may influence normal human sleep.

Subjects were 5 male and 5 female young adult normal volunteers. Females were studied during the follicular phase of the menstrual cycle. Following an adjustment night, each had sleep studies on two nights. On one night they received 100 µg of LH-RH in 500 cc of normal saline intravenously over an 8 hour period starting when the lights were turned out at 11:00 p.m. On the other, randomly-sequenced night they received an equivalent volume of normal saline. The dose of LH-RH, while lower than those reported to produce human sexual arousal, has been shown to acutely raise serum LH and FSH.

Sleep EEG data revealed that LH-RH administration produced no change in the total minutes or percentage of the sleep stages, total sleep, or sleep latency. The subjects were also divided into two groups by sex, and once again there were no significant changes in sleep. It is concluded that low doses of LH-RH, sufficient to stimulate gonadal hormones, have no acute effects on normal human sleep. The possibility remains, of course, that higher doses or more potent analogues of LH-RH may have such actions.

Major Findings: Clinical Disorders

I. EEG Sleep Stages Compared in Normals, Insomniacs, and Depressed Patients.

All-night EEG sleep characteristics of normals, insomniacs, and hospitalized depressed Bipolar and Unipolar Patients were compared. Following an adaptation night, subjects were recorded for three to six nights in the same laboratory setting. All had been drug free for a minimum of two weeks. The depressed patients met criterion of primary affective disorder and were hospitalized because of significant clinical depression at the time of the study. The insomniac patients, who have been described previously, gave a history of significant, prolonged insomnia, did not snore, and were free of significant psychiatric signs, symptoms, or history.

The means were:

	Normals	Insomniacs	Unipolars	Bipolars	ANOVA
Number	41	18	33	23	
Mean Age	41	46	51	41	
Total sleep, min	378	339	286	328	$p<.001$
REM, min	90	86	78	88	
REM Latency, min	78	66	46	46	$p<.001$
Delta, min	24	9	7	16	$p<.005$
Inter. Awake, min	29	40	54	31	$p<.05$
Early Morning Awake	5	9	43	33	$p<.001$

After entering sleep variables into a step-wise, multiple discriminant analysis, 73% of the subjects were correctly classified into their predicted diagnostic group:

Predicted Group Membership (Percentage)

Actual group	Normals	Unipolars	Bipolars	Insomniacs
Normals	100.0	0	0	0
Unipolars	27.3	60.6	12.1	0
Bipolars	13.0	34.8	52.2	0
Insomniacs	22.2	0	16.7	61.1

These results indicate that all disorders of sleep are "not the same," and that all night EEG sleep studies provide reasonably successful classification of depressed Bipolar, depressed Unipolar, and Insomniac patients.

. Depression:

The "Switch Process" in Manic-depressive Illness: Circadian Variation in Frequency of Switches and Changes in Total Sleep Time.

A "switch" was operationally defined as a rapid change in mood (change of three or more points on a 15-point scale) occurring in less than 24 hours.

Out of 75 bipolar manic-depressive patients, 35 showed 70 switches from depression to mania (D→M) and 37 switches from mania to depression (M→D). Next, they were subdivided according to time of occurrence (i.e., which nursing duty shift they occurred in; 11 p.m. to 7 a.m., 7 a.m. to 3 p.m. or 3 p.m. to 11 p.m.). Average sleep time as estimated by nursing staff for five days before (Day -5 to -1) and five days after the day of switch (Day 0 to +4) was calculated. The day of switch was designated as Day 0.

Both D→M and M→D switches occur predominantly in the morning (7 a.m. - 3 p.m.). Among the D→M group, patients who switch at night (11 p.m. - 7 a.m.) show significantly greater sleep loss for the next four days (Day 0 to +4), than if they switch in the morning (7 a.m. - 3 p.m.) ($p<0.01$, Newmann Keuls test). The evening shift does not differ from the other times. The M→D switches, however, fail to show a similar differentiation between the three time shifts with regard to total sleep time. These data indicate that time of switch may predict subsequent sleep changes in D→M switches.

In an unmedicated female bipolar patient fifty-eight all-night polygraphic sleep recordings were obtained during eight switches into and out of mania. While depressed she was hypersomniac and exhibited elevated REM sleep time and short REM latency. On four nights, she apparently switched into mania while asleep. The last recorded sleep stage in each case was REM sleep.

We have analyzed 144 switches (80 switches into mania and 64 out of mania) in another bipolar patient with regular 48-hour manic-depressive cycles. Mrs. J was 41 when she was first admitted to the NIH Clinical Center in 1962 and was hospitalized several times over the course of two years. During her inpatient stay she was studied intensively; the nursing research team rated her every two hours on a 15 point mania scale. Checks were made every fifteen minutes during the night to determine whether she was asleep. We defined a "switch" into or out of mania as a change of manic ratings of at least three points.

Next we tried to localize the specific two-hour time period in which a switch took place using nursing ratings. Switches into mania showed a sharp tendency to occur between 8 p.m. and 8 a.m. with peak incidence between 2 a.m. and 4 a.m. Similarly, switches out of mania occurred significantly more often between 4 a.m. and 6 a.m. than during other time periods. The frequency of distribution of these switches into and out of mania was significantly different from an expected random distribution (2XR chi square analysis with df=11). Switches during sleep: Among the 80 switches into mania 37 occurred out of sleep (41%). Among the 64 switches out of mania, 27 were associated with sleep (42%).

B. The Relationship between Changes in REM Sleep and Clinical Improvement in Depressed Patients Treated with Amiptriptyline

EEG sleep recordings were obtained on consecutive nights from six hospitalized depressed patients before, during, and after treatment with amitriptyline. Amitriptyline significantly reduced time spent in Rapid Eye Movement (REM) Sleep and prolonged the REM Latency throughout the treatment period. Three patients who improved during treatment had a REM rebound when amitriptyline was discontinued, whereas three patients who did not improve showed no REM rebound.

III. Schizophrenia:

A. Schizophrenia

Eight actively ill schizophrenics and eight nonpsychotic controls were deprived of REM sleep by the awakening method for two nights. Sleep patterns during five post-deprivation nights were analyzed by a variety of multivariate techniques.

Data suggest that actively ill schizophrenics are less likely than control psychiatric patients to exhibit a normal REM rebound. They require fewer awakenings than controls to achieve REM deprivation. They show little or no change in REM time or REM% during recovery as compared with baseline, and, compared with controls, have significantly less REM time, REM%, and change in REM time and REM% on early post-deprivation nights.

The two patient groups also differed in their pattern of stages III and IV during recovery. Considerable overlap existed in REM compensation between actively ill schizophrenics and controls. Additional information suggests that REM compensation may be related to Rod and Frame testing: the more field independent a subject is, the better REM compensator he is.

5-Hydroxytryptophan and Schizophrenia

5-Hydroxytryptophan was studied at doses of 200-60000 mg/24 hr with MK485 in 18 chronic schizophrenic patients. EEG sleep studies revealed: (a) REM sleep was reduced at all doses; no rebound was found with 5HTP withdrawal; (b) REM latency was increased at all doses and (c) NREM sleep was increased at low doses, decreased at high doses, and decreased still further during withdrawal.

Absence of Fast Ocular Activity During REM Sleep in Familial Cerebellar Degeneration

The major sleep abnormality in a family with a hereditary cerebeller degeneration was an absolute loss of fast ocular activity during REM. The more affected the individual was with the disorder, the less likely he or she was to display fast ocular movements during REM sleep.

In the absence of neuropathological evidence, it is impossible to precisely characterize the neurologic deficit in this disorder, and, thus, perhaps provide a neuroanatomical basis for the REM's of REM sleep.

Clinically, the disease appears to involve the cerebellum predominately. It is known, however, from animal studies that cerebellectomy does not affect the REM's of REM sleep.

Although vertigo was not a prominent symptom in any of these patients, the electronystagmography results suggest a central vestibular lesion in three patients. While this data is only suggestive, it is consistent with the hypothesis put forward by Pompeiano that REM's depend upon the integrity of the medial and descending vestibular nuclei. These data are consistent with a study by Feinberg who reported diminished REM's in senile dementia.

A Case of Sleepwalking and Somnambulistic Homocide Associated with Psychotropic Drugs

The patient is a 44 year-old white female who had a history of one sleepwalking episode in adolescence but none after that until age 40. She also had had enuresis until age 16. Except for brief psychiatric treatment at age 16 for enuresis and anxiety, she had no psychiatric history until age 39, when she developed a florid psychosis with persecutory symptoms. She was diagnosed as schizophrenic, hospitalized for 2 weeks, and treated with

phenothiazines. A year later she experienced a return of symptoms and resumed treatment on outpatient basis. Because of insomnia, she received thioridazine and Triclos (a chloral derivative) at bedtime. At 3 a.m. on the third night of this regimen, she arose from her bed and stabbed her daughter to death, possibly while asleep or in a confused state. She was found to be hallucinating and delusional when examined the next morning and diagnosed as suffering from paranoid schizophrenia. At her trial she was found innocent on the grounds of insanity and hospitalized under court order.

On phenothiazines and psychotherapy, her psychosis slowly cleared. However, she was noted to sleepwalk 2-4 times per week. Physical investigations, which eventually included EEG's with nasal-pharyngeal leads, a CAT scan, and a pneumoencephalogram, were all normal. Dilantin and tricyclic antidepressants had no effect on her sleepwalking.

After 4 years in the hospital, she was transferred to our unit, and thioridazine 300 mg q.h.s. was discontinued. During a one-month drug-free period, 11 all-night sleep EEG's along with continuous video monitoring were carried out. There were no sleepwalking episodes. However, when treated with thioridazine 300 mg q.h.s. and chloral hydrate 1000 mg q.h.s. she sleepwalked on 5 of the 6 nights recorded. On thioridazine 300 mg q.h.s. alone, she walked one night in four; on chloral hydrate 2000 mg alone, she did not walk during three nights of recording. All sleepwalking episodes occurred out of slow wave sleep.

These results suggest that she sleepwalked only on psychotropic medications, particularly thioridazine. This case is similar to those reported by Huapaya (Am. J. Psychiatry 133: 1207, 1976) of somnambulism secondary to bedtime doses of methaqualone in two cases, chlorprothixene in another, and the combination of methaqualone and thioridazine in the fourth. In all cases sleepwalking could be abolished by either reducing or eliminating the bedtime dose. Two of the cases had childhood histories of sleepwalking, as did our own. As well, Flemenbaum (Am. J. Psychiatry 133:570-572, 1976) has reported cases of pavor nocturnus, another disorder associated with stage 4 sleep in patients receiving large bedtime doses of tricyclics or antipsychotics. The mechanism of this phenomenon, which may not be uncommon, is unknown. We are presently analyzing our patients' sleep EEG's.

Major Findings: Normal Studies

I. Effects of Sleep Stage and Stimulus Intensity on Auditory Evoked Responses

The purpose of this study was to evaluate the effect of sleep stage and stimulus intensity on the auditory evoked response (AER). Nine normal volunteers (age 18-22; 6M, 3F) slept in the sleep lab for three nights each; the first night was adaptation and was not included in any calculations. Four intensities of clicks (50, 60, 70 and 80 dB) were

generated by the LINC computer in pseudo random order. EEG was sampled at 250 Hz for 1 second and AER was computed on line for each intensity for each minute of the night. Each minute was visually scored for sleep stage. AER from the minutes of each sleep stage were then averaged. AER amplitude tended to increase little from 50 to 80 dB in waking subjects but increased markedly in sleeping subjects in stages 3 and 4 ($p<0.05$, 2-way analysis of variance). Rapid eye movement (REM) and stage I sleep had small amplitude AER's in comparison with other sleep stages.

Individuals who showed decreases in amplitude at high intensities while awake slept significantly longer during the experimental night ($r=-.76$, $p<0.05$). The loss of inhibitory mechanisms during stages 3 and 4 is well known. It is therefore not surprising to see the disappearance of the paradoxical AER amplitude reduction at high intensities and a more linear EP amplitude/stimulus intensity function; stage 1, 2 and REM sleep resembled the waking state. Of interest was the finding that waking AER amplitude/stimulus intensity functions predicted sleep duration in this noisy environment. This suggests that individual differences in sleep duration may be related to differences in inhibition of sensory processing.

r Findings: Animal Behavior

Deprivation of Rapid Eye Movement Sleep and Nesting Behavior in Rats

The effects of REM deprivation for four days on nesting and locomotor activities were studied in male rats. Four groups of animals were tested. (a) Experimental animals (SP), deprived of REM on a small (6.5 cm) pot; (b) Large pot (LP) control, maintained on large (12.5 cm) pots; (c) stress group, forced swimming for 1 hr/day; (d) normal controls. The results indicate, as predicted, increased utilization of nesting material by the experimental (SP) group in the post-REM deprivation period as compared with normal controls (464 cm for SP, 94 for normals, $p<.05$). Nevertheless, the SP group did not differ significantly from LP (356 cm) or stress (133 cm). Since LP does suffer some REM deprivation, though less than SP, the results suggest that increasing levels of REM deprivation may increase a drive-related behavior in rats.

EEG Documentation of a Method for Sleep Depriving Rats

A variety of methods have been employed to produce total sleep deprivation in small animals. These include continuous handling, the use of treadmills or small platforms surrounded by water and administration of amphetamines or electric shocks. Unfortunately, these techniques are largely without support of EEG data. Most also have clear disadvantages. The use of small platforms surrounded by water is an outgrowth of a procedure for REM sleep deprivation; when used for purposes

of total sleep deprivation it actually may cause a profound suppression of REM sleep with a smaller decrease in NREM sleep, which still occurs. Other approaches, such as continuous handling of the animals, require enormous amounts of labor. There is a need, then, for a relatively simple method of sleep deprivation which is supported by EEG data and which can process a number of animals at once with a minimum of labor. In this abstract we shall describe such a technique, which consists of keeping rats awake by maintaining them in slowly rotating cylinders.

Rats were placed in two Nalgene cylinders (68.6 x 35.6 cm), each of which is divided into six compartments. An electric motor causes them to rotate at 3 RPM. A strip of tubing on the floor of each compartment provides an obstruction over which rats must walk, so that they cannot slide along.

In order to document the state of consciousness of rats undergoing this procedure, we recorded the EEG and EMG of four rats in the moving cylinders for 24 hours and compared them to seven animals in a stationary cage. The moving cylinder reduced sleeping time from 47.0% of the 24-hour period to only 3.8% ($p<0.001$). The small amount of sleep which did occur was made up of both REM and NREM sleep. The percentages of total sleep time did not differ between the two conditions. Thus, there was no selective effect on one particular sleep stage, and instead total sleep time as a whole was markedly reduced.

We have presented a method of reducing total sleep to less than a tenth of normal for prolonged periods of time. Unlike the modified use of the platform surrounded by water, it does not totally suppress one sleep state (REM) while only partially suppressing another (NREM sleep). A much larger number of rats (12) can be studied at one time than in previous procedures. It is anticipated that this will be useful in observing the effects of sleep deprivation in studies dealing with memory, neurochemistry, and possible circulating sleep factors.

EEG ANALYSIS OF RATS IN SLEEP-DEPRIVATION APPARATUS FOR 24 HOURS*

	$\frac{\text{TOTAL SLEEP TIME}}{\text{TOTAL RECORDING TIME}} \times 100$	$\frac{\text{REM SLEEP TIME}}{\text{TOTAL RECORDING TIME}} \times 100$	$\frac{\text{NON-REM SLEEP TIME}}{\text{TOTAL RECORDING TIME}} \times 100$
Control (n=7)	47.0 $\pm$ 3.8	7.4 $\pm$ 0.5	39.6 $\pm$ 3.7
Experimental Group (n=4)	3.8 $\pm$ 1.9	0.2 $\pm$ 0.0	3.6 $\pm$ 1.9
Significance**	$p < 0.001$	$p < 0.001$	$p < 0.001$

* Values represent means $\pm$ SEM
** Two-tail t-test

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00098-04 BP

PERIOD COVERED
October 1977 to September 1978

TITLE OF PROJECT (80 characters or less)
Study of Children and Grandchildren of Patients with Depressive Illness

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	J. R. Hamovit, MSW	Research Social Worker	BP	NIMH

COOPERATING UNITS (if any)

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Biological Psychiatry Branch

SECTION
Unit on Childhood Mental Illness

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	1.4	1.1

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☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

It is the long range purpose of this project to determine the frequency of affective disorders in the children of adults with unipolar and bipolar depressive illness, and to identify biological markers of a genetically-transmitted predisposition to affective disorders, and to study the interaction between genetic predisposition and environmental factors in children with affective disorders. Our present project is a refined replication of our pilot project reported last year. The major changes involve the inclusion of two control groups as well as a blind interviewer and rater.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: (1) to determine the frequency of affective disorders in the children of adults with unipolar and bipolar depressive illness, (2) to identify biological markers of a genetically-transmitted predisposition to affective disorders, and (3) to study the interaction between genetic predisposition and environmental factors in children with affective disorders.

Methods Employed: We are studying three groups of children: (1) 50 children of parents hopsitalized at NIMH with a primary affective illness, (2) 25 children of patients hospitalized at NIH because of a physical illness and screened for any affective disorder, and (3) 25 children of "normal" parents with no known physical or mental disorder. All children are seen twice, four months apart. After the second interview, blood is drawn on the 50 children in group 1 for determination of genetic markers as well as blood serotonin levels (in cooperation with Drs. Elliot Gershon and Dennis Murphy).

A social worker obtains at each visit a history from the parents and administers the Achenbach Scale for childhood psychopathology.

At each visit, the child has a psychiatric interview. The interview is conducted by a blind interviewer and rater, while another investigator observes through a one-way mirror. The interviews are structured, and follow a standard inventory of topics aimed at eliciting various types of psychopathology such as affective disorders, anxiety reaction in childhood psychosis, delinquency and minimal brain dysfunction. A portion of the interview is aimed at eliciting fantasy material by the use of free drawings, story telling, and free reporting of dreams, T.V. stories, and movies. Other methods used in child psychiatry such as the three wishes and the Despert fables are also employed. Following the interview, the children are rated on the CPRS scale of the ECDEU as well as on a global rating scale for affective disorders constructed for this purpose and used by the investigators for many years. All the above mentioned procedures pose no hazards for the children. In addition, after each visit, the children will be rated on the Weinberg scale.

Major Findings: In our pilot study of the 30 children, seven fell into the severe range and nine into the moderate range on a depression score devised by totaling the affective rating items in the various scales we used. None of the children were hypomanic or psychotic and there was no correlation between the depressive symptoms and the sex and age of the children. In our current project, we have seen so far 48 subjects as well as their parents. A preliminary analysis of our data on 48 children was done under the direction of Dr. John Bartko. (He employed safeguards to maintain the blind.) The results so far reveal significant differences of the presence of a depressive disorder between the probands and the control groups. A further analysis is still in progress.

Significance to Biomedical Research and the Program of the Institute: There is much interest in determining whether affective disorders can be rated to predict biochemical and other physiological measures which may be genetically determined. Also, there is a considerable interest presently in the nature and prognosis of affective disorders in children and their relationship with affective disorders in adults. It is to be hoped that our present study will help to clarify some of the issues mentioned above.

In addition to the potential significance of clarification of the problem of affective disorders in children, this study has the potential of developing preventive measures in children and perhaps (psychotherapeutic and pharmacological) measures to reduce and forestall, or reduce the frequency of, affective disorders in adults.

Proposed Course of Project: (A) We plan to continue our on-going project until all the 100 children have been seen. Following this phase of study, the analysis of all the available data will be done. The decision whether we will see all the 100 children as originally proposed will depend on the outcome of our current data analysis. (B) (1) We plan to continue to follow these patients twice a year for as long as possible. At each follow-up visit, there will be a history given by the parents, using the Achenbach Rating Scale, a psychiatric interview and rating of the child 2) The parents of all the children will be seen in a conference and the findings will be discussed. When significant psychopathology is found, the family will be counselled about securing psychiatric help for the child. (C) We plan a pharmacological study in which depressed children of Lithium responders will be given Lithium and placebo in a crossover design. Dr. John Bartko serves as a consultant in the design of this project.

Publications: A paper titled "Offspring of Patients with Affective Disorders", was accepted for publication in the British Journal of Psychiatry and is now currently in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00100-03 BP

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)
Psychological and Biological Aspects in Childhood and Adolescent Mental Illness

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	C. L. Ludlow, Ph.D.	Speech Pathologist	CDP	NINCDS

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
5.42	2.82	2.60

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☒ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

An inpatient program with selected overnight stays, for childhood and adolescent neuropsychiatric disorders is ongoing. The conditions currently under study are hyperactivity and Gilles de la Tourette's Syndrome. Pharmacological compounds under study in these disorders include methylphenidate, amphetamine, piribedil, Clozapine, and haldol. Pharmacokinetic studies with behavioral correlations are on-going. Central neurotransmitters and nucleotides, and their metabolites are being studied in plasma and urine. Early findings indicate that the amphetamine half-life in children is about 1/3 that of adults. Behavior and motor activity response do not correlate with plasma amphetamine levels. These responses occur during the absorption phase as determined by serial plasma amphetamine following a single dose.

Project Description:

Objectives: The objectives of this program are broad. In the most basic sense, the objective is to gain new knowledge of the central nervous system functioning of children and adolescents with especial reference to maturational changes. Compared to the neurobiological knowledge that has been gathered related to adult neuropsychiatry, much less has been gathered in relation to neuropsychiatric disorders in children. An integration of this developing neurobiological knowledge and childhood neuropsychiatry is a pressing need.

Several principles are important to keep in mind in the study of children versus adults: 1) morphological brain maturation is age related, i.e, the reticular activating system, of considerable importance in psychogiological studies, is not fully mylinated until ages 10 to 20; 2) children may have more rapid and dramatic pharmacological responses, both therapeutic and toxic; 3) no clear history of pre-adolescent "addiction" to compounds, i.e, amphetamine, which can effect unequivocal addiction in adolescents; 4) most behavioral symptoms in children that show pharmacological responsivity have more of an apparent organic contribution than those of adults, i.e., motor hyperactivity, enuresis, poor attention span in children verus complex emotional and cognitive aberrations in adults; 5) clinical symptomatology is more variable and fluctuating in children than usually seen in adults, i.e., rating scales for endogenous depression in adults are more easily constructed to measure a "constellation" than is the case for childhood depression.

An objective in support of the above is to elucidate the mechanisms of actions of pharmacological compounds in children and utilize new compounds when appropriate. In addition, a number of studies are being undertaken to elucidate cerebral metabolism in specific disorders and possible protein defects in those disorders.

Methods Employed: An inpatient program for children, and adolescents, involving selected over-night stays, has been established on the 2-West Nursing Unit. Children who are hyperactive, aggressive and impulsive, and who have learning difficulties have been admitted in order to study a more closely defined population of hyperactive children. In addition, patients with Gilles de la Tourette's syndrome are also being studied. There are future possibilities for studying children with autism and various metabolic defects in conjunction with other institutes.

All children are thoroughly evaluated by pediatric, medical, neurological and psychiatric examinations with all routine and other indicated procedures and laboratory studies. Children are also administered various psychological and neuropsychological tests and receive a psycholinguistic examination in a collaboration with NINCDS. Neurological examinations are scored carefully according to a rating scale (PANESS).

The problem of a reliable diagnostic and nosological system in child psychiatry is of paramount importance. A parents' questionnaire developed by Achenbach which divides children into nosological groupings by factor analysis is being scored blindly on all admissions and will be compared to those diagnoses ascertained in the usual clinical way and by research rating instruments specific for a disorder (as hyperactivity).

Pharmacological compounds, both standard and those previously unused in children are being studied. Serial plasma pharmacokinetic data are being generated for methylphenidate and amphetamine. These data are studied in conjunction with motor activity, behavior, and cognition, the methodology for which is described below. Piribedil, a specific dopamine (DA) agonist, is being given in an early Phase II trial in hyperactive children and clozapine, a neuroleptic, is likewise being given in Gilles de la Tourette's syndrome. L-Dopa, a precursor of DA is being administered to hyperactive children as a clinical trial.

Motor activity is measured by an ambulatory activity monitor with solid state memory which measures individual motor movements via a pendulum acceleration system per unit of time and set at a desired sensitivity for the particular study. The unit contains a memory bank that stores this data for 40 hours. At any time the instrument can be read into a computer for a print-out in numerical form or in graph-form. Behavioral changes in hyperactive children are measured via Conners Teachers' Rating Scale (CTRS). The abbreviated 10-item scale is scored daily by a teacher in a classroom that is an integral part of the study program. On days of serial plasma amphetamine determinations, these behavioral changes are measured hourly. Cognition is measured by a continuous performance task in which errors of omission and comission can be scored in terms of differing time intervals. Time intervals can also be increased or decreased in relationship to accuracy of response.

Amphetamine is being measured by a radio immunoassay. Biochemical studies include 24-hour urine collection to study homovanillic acid (HVA) and 3-methoxy-4-hydroxyphenylglycol (MHPG). These determinations are divided into day and night collections. Plasma half-lives of pharmacological compounds are being ascertained. Plasma norepinephrine (NE) changes as they relate to plasma amphetamine levels have been studied.

Neurophysiological studies include routine and sleep EEG's and EMI scans when indicated. Average Evoked Response (AER) studies are being conducted as they relate to hyperactive children in drug-free and treated conditions. Psycholinguistic changes are also studied in relation to amphetamine effects.

Major Findings:: The evaluations of data regarding diagnoses and various psychological and behavioral findings are largely preliminary; however significant correlations exist between community teachers' and NIH teachers' CTRS ratings of behavior.

Serial plasma pharmacokinetic data indicates that children reach a peak serum level of amphetamine within 3-4 hours of an initial dose; however as much as 70-80% will remain in the serum at 5-6 hours when behavioral effects have largely dissipated. Apparent elimination half-lives are about 6 hours. Test-retest studies of individuals indicate that both pharmacokinetic data and motor activity (behavioral) and response data has also been studied after use of sustained release capsules. Socially appropriate behavioral change, increased attention span and concentration ability is maximal at 1-3 hrs. after administration of a single dose (0.5 mg/kg) of d-amphetamine during its absorption phase. This data indicates that the clinical changes may be related to depletion of catecholamine stores, to replacement by a "false neurotransmitter metabolite of amphetamine, or to alteration in receptor sensitivity.

Study of urinary metabolites indicate that day and night excretion of MHP and HVA are not significantly different; however d-amphetamine after 8 days is associated with significantly lower MHPG levels and some evidence indicating that behavioral response is associated with the decrement in MHPG.

Hyperactive children are not significantly different from normal controls with regard to plasma NE and dopamine-B-hydroxylase (DBH) but do have significantly more neurological soft signs by PANESS examination. Plasma NE is significantly correlated with anxiety and hyperactivity (CTRS, Factor IV) ratings and with the increased plasma levels of d-amphetamine. Plasma NE itself is significantly elevated at 2-3 hours following a single oral dose of d-amphetamine.

Studies evaluating continuous cognitive performance are now under way, but results are preliminary. Electroencephalographic and AER studies are also preliminary. Data from psycholinguistic evaluations are being prepared for publication.

Significance to Mental Health Research: Though childhood neuropsychiatric disorders, and particularly hyperactive children, have been considerably studied in the last few years, there are many diagnostic and psychopharmacological questions to be answered. The majority of studies in the past in child psychiatry have been related to psychological, psychodynamic issues. As regards hyperactive children, Gilles de la Tourette's, psychoses, and autism, an increased interest in psychopharmacology has emerged. Though methylphenidate and amphetamine give positive responses in 80% of well diagnosed hyperacative children; and, likewise, haldol gives a high frequency of positive response in Gilles de la Tourette's syndrome, the pharmacokinetics and metabolism of these drugs have not been closely studied in children. The correlations between such basic pharmacological knowledge and clinical effects have also been under-studied in children in general. More importantly, for the future, basic biological factors in childhood neuropsychiatry which might elucidate the psychopharmacological responses

are, at this point, only hypotheses. The degree to which these hypotheses are validated or refuted could play a significant role in understanding the biological contributions to childhood neuropsychiatry.

Proposed Course of Project: We plan to conduct this program over an indefinite period.

Publications

Caine EC, Margolin D, Brown GL, Ebert MH. Gilles de la Tourette syndrome tardive dyskinesia and psychosis in an adolescent. Am J Psychiatry 135: 241-243, 1978

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Rapoport JL, Mikkelsen EJ, Ebert MH, Brown GL, Weise VK, Kopin IJ: Urinary catecholamines and amphetamine excretion in hyperactive and normal boys, J Nerv Ment Dis, in press, 1978

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00101-02 BP

PERIOD COVERED

October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Childhood Enuresis: Clinical Response to Placebo, Imipramine and Methscopolamine

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	R.J. Wyatt, M.D.	Chief, Lab. of Clin. Psychopharmacol.	SMR	NIMH
	W.E. Bunney, Jr., M.D.	Chief, Biological Psychiatry Branch	BP	NIMH

COOPERATING UNITS (if any)

Laboratory of Clinical Psychopharmacology, NIMH

LAB/BRANCH

Biological Psychiatry Branch

SECTION

Unit on Childhood Mental Illness

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.65	.40	.25

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☒ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A series of studies are planned to investigate severe enuresis in males aged 6-12 using electroencephalographic measures during treatment with placebo or active drugs thought to benefit the condition. Half of the subjects are selected to have behavioral disturbance while half have enuresis as a single symptom. The state of sleep during which enuretic events occurred were recorded as well as other sleep parameters. At the present time 40 children have been studied.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: The purpose of this first study is to elucidate the mechanism by which imipramine is clinically effective in enuresis. A second purpose is to compare the sleep patterns of behaviorally disturbed and non-behaviorally disturbed children at baseline and during treatment with imipramine, an antidepressant in general use for a variety of childhood disorders. Enuresis has a strong association with childhood psychiatric disorder although it also occurs as an isolated symptom. It is hoped that through the study of the mechanism by which imipramine acts, and of the sleep patterns of these patients, the underlying pathophysiology of the disorder will be better understood.

Methods Employed: Patients were initially selected by telephone screening on the basis of the severity of the enuresis, and whether the child could be clearly identified as belonging in one of the study groups, i.e., with associated behavioral disturbance, or with enuresis as an isolated symptom. The Peterson Quay behavior checklist was used for this purpose. On admission to ward 2-West, a medical, neurological and psychiatric examination was carried out. Three nights of baseline sleep recordings were made. Utilizing a structured psychiatric interview for use with children, a diagnostic evaluation is made during the baseline period. Following three nights of baseline sleep recording, the children are given three sequential outpatient trials of medication. The first period is a single blind placebo trial, followed by a double blind cross over trial of methscopolamine (6 mgm hs) or imipramine (75 mgm hs). At the end of each 2 week trial period, sleep recordings were repeated and blood samples obtained for blood level of imipramine and desmethylimipramine.

Major Findings: Forty-two subjects have been studied to date; there have been two dropouts from the study. The most striking finding from baseline evaluation was that enuretic events occur during all sleep stages and not, as had been previously reported, primarily during slow wave sleep. A) No subject responded to placebo. B) There was a significant difference between the efficacy of the two active drugs; imipramine produced a significant decrease in enuretic episodes while methscopolamine did not. These findings indicate that imipramine action in enuresis is central rather than peripheral, as methscopolamine has only central anticholinergic activity.

Significance to Mental Health Research: As enuresis is strongly associated with other psychiatric disturbance of childhood, a study of the pathophysiology of the condition may shed light on more general physiological disturbance. The comparison between disturbed and non-disturbed enuretic children may differentiate two separate conditions, an "arousal" disorder occurring in Stage II-IV sleep more common in behaviorally normal children and a disorder related to "lighter" sleep stages more common in behaviorally disturbed children. Methscopolamine has peripheral anticholinergic action but does not cross the blood brain barrier. The failure of methscopolamine

to be effective in enuresis supports a central action of imipramine although other peripheral effects (such as noradrenergic potentiation) cannot be ruled out. The data from the first study with 14 children appears sufficiently clear. Therefore, the project has been amended and a new study (Project Number 76-M-384-A) will be conducted to further elucidate the mechanism of action of imipramine in this condition (see below).

Proposed Course of Project: The findings of this first study, that methscopolamine is not clinically effective in enuresis, suggests a series of studies to pursue the action of imipramine in this condition. Two studies are planned. The first will be a comparison of desmethylimipramine and imipramine treatments of enuresis. The reason for this comparison is that DMI is relatively selective in its central effect of blocking the reuptake of norepinephrine while imipramine has equal potency in blocking both norepinephrine and serotonin uptake. DMI has approximately half the central and peripheral anticholinergic potency of imipramine. If DMI and imipramine are equally effective clinically, it is probable that the central therapeutic serotonergic or cholinergic mechanisms are implicated; a second study is planned to further dissect the enuresis syndrome. This study will investigate the antienuretic effects of Dalmane, a potent inhibitor of slow wave sleep. A leading theory of the cause of enuresis is that it is a disorder of "arousal". If this is correct, Dalmane should be effective in the treatment of enuresis, at least for those individuals whose enuretic episodes occur primarily during state II-IV sleep. The individual diagnostic profiles of the children will be examined in relation to response to each of the above agents. It is possible that each agent will be useful for a specific type of enuresis and that the syndrome may prove a heterogeneous disorder.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00102-1 BP

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)
Lithium Response in Children of Lithium Responding Manic Depressive Parents

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	Ronald Reider, M.D.	Staff Psychiatrist	LP	NIMH
	Monte Buchsbaum, M.D.	Chief, Unit on Perceptual and Cognitive Studies	BP	NIMH
	William E. Bunney, M.D.	Chief	BP	NIMH
	Judith L. Rapoport, M.D.	Staff Psychiatrist	BP	NIMH
	Elliot Gershon, M.D.	Unit Chief, Section on Psychogenetics	BP	NIMH
	J. R. Hamovit, MSW	Research Social Worker	BP	NIMH
	W. Sceery, D.J.	Social Worker	BP	NIMH

COOPERATING UNITS (if any)

Laboratory of Psychology and Psychobiology, NIMH

LAB/BRANCH
Biological Psychiatry Branch

SECTION
Unit on Childhood Mental Illness

INSTITUTE AND LOCATION
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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.6	1	0.6

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The effects of Lithium in emotionally disturbed children having at least one manic-depressive lithium responsive parent will be examined. Thirty children, ages 5-15, of both sexes, having psychiatric symptoms that are in some way incapacitating and are of major concern to the child, parent or teacher, will be selected. Children will be given lithium and placebo, in a double-blind, crossover fashion for 16 weeks. Behavior ratings will include: parent's and teacher's assessment, psychophysiological measures, child interview and cognitive testing. The lithium administration will be monitored by an "unblind" coinvestigator.

Project Description:

Objectives: 1) To test the hypothesis that some of the offspring of lithium responding parents will prove clinically responsive to lithium. 2) To test the hypothesis that children with an affective and/or cyclic component to their psychopathology will be better responders. 3) To see if the response to lithium may possibly be a genetic marker for manic-depressive illness. 4) To identify the children at risk with early prodromal disorders who respond to lithium, prior to the outbreak of manic-depressive illness.

Methods Employed: The 30 patients will be admitted to the study upon recruitment from the NIMH Outpatient Department (OPD) as well as from the local psychiatric community. The fact that the parents are indeed lithium-responding manic-depressives will be independently verified on the basis of direct examination and medical records of parents.

The patients will be selected on the basis of having sufficient psychopathology for it to be incapacitating and to be of concern to their parents and/or the school authorities. Any children with significant brain damage and/or an IQ below 80 will be excluded from the study. The patients will be ages 5-15, of both sexes, regardless of their socio-economic status. These patients will be admitted to the Outpatient Clinic for the duration of the study (16 weeks). Patients of special interest may be retained in the OPD for follow-up treatment and study. Other patients will be returned to their previous source of care or referred to local psychiatrists, as needed.

Their work-up records will be handled as described in the Handbook for Staff Physicians.

Admission Work-Up:

Parents: A structured history will be obtained. In addition, the parents will be requested to fill out the following questionnaires: 1) Deviant Behavior Inventory (DBI), 2) The Achenbach Scale, and 3) The Conner's Parent Questionnaire;

Patients: A diagnostic evaluation will be obtained including a structured interview and a routine physical exam. On the basis of all available information, a clinical diagnosis based on DSM-III will be made. Any affective component will be evaluated using the Weinberg criteria for depression and mania (13,14). The following rating scales will be filled out: 1) Children's Psychiatric Rating Scale (CPRS of the ECDEU, 2) Periodicity Rating, and 3) Children's Affective Rating Scale (CARS). In addition, a brief battery of projective psychological tests will be administered (T.A.T., Rorschach, a Bender-Gestalt Test, and a Continuous Performance Test-CPT).

Blood will be drawn for BUN and T_3, T_4 to rule out renal or thyroid pathology. These tests will be repeated at the end of the study.

The following physiological tests will be administered: 1) Average Evoked Response (AER), and 2) A routine EEG. The EEG will help us to exclude children with brain abnormalities. The AER may help to predict lithium responders who have a greater visual AER (16), termed augmenting.

Teachers will fill out the full Conners' Teacher Rating Scale (CTRS).

The thirty patients will be randomly assigned to 2 groups (I and II). Group I will be treated as follows: 4 weeks placebo, 4 weeks lithium, 4 weeks placebo, 4 weeks lithium. The procedure will be reversed in Group II.

Nature of Procedures: Lithium will be administered in blind fashion except that a case "coordinator" will be unblind. The first lithium administration will begin with the mean child dose for that age and will be adjusted until a blood level between .8 and 1.2 mg/liter is obtained. The case coordinator will adjust the number of tablets for a matched placebo case in parallel with adjustment for active drug.

Side effects will be monitored by using a lithium side effect checklist (see Appendix I). The second period of lithium administration will begin with that child's effective dose.

1) The patients will be seen on a weekly basis for evaluation and administration of medication. At each visit the following procedures will be carried out: A brief structured interview of 15-30 minutes with a special focus on the target symptoms. The interview will be rated on the target items of the CPRS and the CARS. Lithium levels will be obtained both in serum and saliva. The Side Effect Checklist will be filled out by the "unblind coordinator" who will also adjust the dosage at that time.

2) At the 4, 8, 12, 16 week visits the following procedures will be carried out:

Patients: A full structured interview, a full CPRS, CARS and CPT. Also, the AER will be repeated at these times.

Parents: A full DBI and Achenbach Scale.

Teachers: A full Conners' Teacher Rating Scale.

Major Findings: None to date.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00151-01 BP

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)
Behavioral and Electrophysiological Response of Normal Children to a Single Dose of Dextroamphetamine

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. L. Rapoport, M.D.	Staff Psychiatrist	BP	NIMH
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	C. L. Ludlow, Ph.D.	Speech Pathologist	CDP	NINCDS
	H. Weingartner, Ph.D.	Research Psychologist	LP	NIMH
	M. H. Ebert, M.D.	Acting Chief, Sec. on Experimental Therapeutics	LCS	NIMH
	I. J. Kopin, M.D.	Chief, Laboratory of Clinical Science	LCS	NIMH
	W. E. Bunney, Jr., M.D.	Chief, Biological Psychiatry Branch	BP	NIMH

COOPERATING UNITS (if any)

Laboratory of Psychology & Psychopathology, NIMH; Laboratory of Clinical Science, NIMH; Section on Communicative Disorders, NINCDS

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Biological Psychiatry Branch

SECTION
Unit on Childhood Mental Illness

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.00	.75	.25

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this amendment was to study the effects of a single dose of dextroamphetamine on normal adult males. The initial protocol established the fact that normal children show a response to amphetamine qualitatively similar to that of hyperactive children. The amended protocol will investigate the response of normal adults to the identical tasks used in hyperactivity research.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: 1. To determine the nature of response of normal adults to dextroamphetamine in the test situation usually employed in hyperactive child research. 2. To correlate ratings of childhood and adult hyperactivity with response to stimulant within the normal adult sample.

Methods Employed: During the period covered 30 normal adults will be examined at baseline and during a double-blind crossover administration of either placebo or dextroamphetamine. One group will receive 0.5 mg/kg and a second group will receive .25 mg/kg. All subjects are admitted to Ward 2-West and receive a test battery including reaction time and GSR, Continuous Performance Test, Paired Associate Learning and Porteus Maze Test. Motor activity is recorded during the test session.

Major Findings: To date, 14 normal males have been tested who received the 0.5 mg/kg dose. Ten of the subjects moved less during testing while on drug while 4 moved more. There is an increased vigilance, decreased reaction time and improved learning of verbal material. These changes are similar to those seen with hyperactive children for the most part. However, as the absolute dose given this group is rather large (35 mg/subject), a second group will be tested which receives a lower dose of stimulant.

Significance to Biomedical Research and Program of the Institute: The diagnostic significance of a beneficial response to stimulant drugs is of major interest both to child psychiatry and general psychiatry. If hyperactive children respond to stimulants in an indentical fashion to normal children, then it is not necessary to postulate hypoarousal of dopamine deficiency as a necessary basis for clinical response. Moreover, some animal models of hyperactivity are now seen as erroneous as these employ animals who become excited following stimulants unless chemical or anatomical lesions are made. The current protocol suggests that this is an invalid model for clinical hyperactivity.

The adult subjects are all judged to be currently free from psychiatric illness: however, they vary widely with respect to retrospective reports of childhood behavior. The relationship, if any, between childhood history and adult behavioral response to stimulant will be relevant to the growing interest in the use of stimulant drug treatment in "Adult Minimal Brain Dysfunction."

Proposed Course: It is anticipated that the project will be completed during the period covered in this report.

Publications:

Rapoport, J, Buchsbaum, M, Weingartner, H, Zahn, T, and Ludlow, C: Dextroamphetamine: behavioral and cognitive effects in normal prepubertal boys. Science 199: 560-563, 1978

Ludlow, C, Rapoport, J, Cardano, C, and Mikkelsen, E: The differential effects of dextroamphetamine on the language and communication skills of hyperactive and normal children. In Knights, R, and Bakke, H (Eds.): Learning Disabilities -- 2nd NATO Conference. Baltimore University Park Press, 1978, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00152-01 BP

PERIOD COVERED
March 20, 1978 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Effects of Sinemet on Behavior in Hyperactive Children

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Judith L. Rapoport, M.D.	Staff Psychiatrist	BP	NIMH
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	Michael Ebert, M.D.	Chief, SET	LCS	NIMH
	Edwin J. Mikkelsen, M.D.	Clinical Associate	BP	NIMH
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COOPERATING UNITS (if any)
Laboratory of Clinical Science, DCBR, NIMH

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Biological Psychiatry Branch

SECTION
Unit on Childhood Mental Illness

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.65	.40	.25

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A double blind crossover study is planned to evaluate the clinical response of approximately 15 hyperactive boys to Sinemet (maximum dose, 60mg/600mg). The behavioral, cognitive responses will be correlated with changes in plasma norepinephrine and measures of dopamine turnover.

Project Description:

Objectives: Hyperactive children usually respond symptomatically to stimulant medications which are known to increase the concentration of monoamines at the receptor sites in the brain. As L-dopa is known to increase the availability of dopamine in the brain, if a deficiency of dopamine were responsible for at least some cases of hyperkinesis, then L-dopa should be expected to have some therapeutic value.

Methods Employed: The recent practice of combining L-dopa and Carbidopa, a peripheral dopa decarboxylase inhibitor, lowers the necessary dosage and thereby decreases side effects making this treatment more feasible for children. Hyperactive boys, aged 6-12, exhibiting restlessness and impulsivity both at school and during an inpatient evaluation period will be selected for the protocol. Evaluation will include medical, neurological, and psychiatric examination as well as psychological testing.

Children will be started on a gradually increasing dosage of Sinemet or placebo; medication will be increased over two weeks until the maximum dose of 6 mg/600 mg Sinemet or 6 tablets of placebo will be given during the third week as tolerated.

During the third week of placebo or Sinemet treatment, plasma norepinephrine and HVA turnover will be assessed. Clinical response for the group will be compared for drug and placebo periods and individual responses will be related to changes, if any, in the catecholamine measures.

Major Findings: The project will begin June, 1978.

Significance to Mental Health Research: As hyperactivity may be a forerunner of adult sociopathy, alcoholism or schizophrenia, studies on treatment and underlying pathophysiology of hyperactive children have wide application throughout the field of mental health research. Alternate treatments to stimulants are practically useful as approximately 15% of hyperactive children do not respond favorably to stimulants.

Publications: None.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00153-01 BP

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

The Treatment of Obsessional Children with Chlorimipramine

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	W. E. Bunney, Jr., M.D.	Chief, Biological Psychiatry Branch	BP	NIMH

COOPERATING UNITS (if any)

Neuropharmachology Branch, LCS, NIMH

LAB/BRANCH
Biological Psychiatry Branch

SECTION
Unit on Childhood Mental Illness

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.25	.10	.15

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Obsessional disorder of childhood is a rare disorder about which little is known. The purpose of this protocol is to collect basic data on family history of mental illness, sleep measures and neuropsychological testing on a selected group of children and adolescents with obsessional disorder. A drug trial of chlorimipramine, desmethylimipramine and placebo will be carried out to evaluate the effect of antidepressant and the specificity of chlorimipramine for this disorder.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: To evaluate the clinical response of Obsessive-Compulsive disorder of childhood to chlorimipramine and to relate this response to changes in ratings of depression. Clinical response to chlorimipramine will be related to platelet serotonin at baseline.

Methods Employed: Patients are sought on a national level because of the rarity of the disorder. Inclusion criteria are Obsessive-Compulsive disorder as a primary disturbance. Children must have IQ of 85 or above, and be free from known neurological disturbance or psychosis.

A modification of the Leyton Obsessional Inventory will be used to monitor drug effects on Obsessive-Compulsive symptomatology throughout the 16 week clinical trial. Weekly ratings are made by two physicians and ward nurses on the CPRS.

Plasma levels of drug as well as platelet serotonin will be assayed for each drug phase.

Major Findings: Three children have entered the protocol: 2 males aged 14 and one 13 year old female. The first patient improved gradually throughout his hospital course and did not have a different course during chlorimipramine treatment. Depression ratings changed in parallel with obsessive symptoms. The second two patients have not yet completed the protocol.

Preliminary analysis of family pedigree data indicates high incidence of depression among first degree relatives. Sleep recordings on subject No. 1 showed marked decrease in REM latency at baseline which increased over time with clinical improvement. These findings indicate a possible biological link between depression and obsessive-compulsive disorder in childhood.

Proposed Course of Project: The findings from this first study prompted an amendment to the study and the second 20 subjects studied received either imipramine or desmethylimipramine (75 mg hs) or placebo. The reason for this comparison is that DMI is relatively selective in its central effect of blocking the re-uptake of norepinephrine while imipramine has equal potency in blocking both norepinephrine and serotonin uptake.

Preliminary results suggest that imipramine is slightly more effective than DMI in antienuretic effect although both drugs are significantly better than placebo in preventing enuretic episodes. The relative superiority of imipramine implicates cholinergic or serotonergic mechanisms as at least part of the beneficial action of imipramine. The data will be analyzed to examine clinical characteristics of the patients in relation to differential response to the two drugs. In addition, plasma level of tricyclics will be assayed and related to clinical efficacy.

Publications:

1) Rapoport J, Zavadil A, Mikkelsen E: Plasma tricyclic concentration and clinical antienuretic response. Psychopharmacol Bull. 1978, in press
2) Rapoport J: Antidepressant drug use in child psychiatry. Affective Disorders -- America's Public Health Problem, Ayd F (ed). F Ayd Publishers, Baltimore, 1978, in press

MITHSONIAN SCIENCE INFORMATION EXCHANGE ROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00034-08 BP

ERIOD COVERED
October 1, 1977 through September 30, 1978

ITLE OF PROJECT (80 characters or less)
Psychological and Physiological Correlates of the Average Evoked Response

AMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER ROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. S. Buchsbaum, M.D.	Chief, Unit on Percept. and Cognitive Studies	BPB NIMH
OTHER:	R. Coppola, D.Sc.	Electrical Engineer Unit on Perceptual and Cognitive Studies	BPB NIMH

OOPERATING UNITS (if any)
None

AB/BRANCH
Biological Psychiatry Branch

ECTION
Office of the Chief, Unit on Perceptual and Cognitive Studies

NSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

OTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	0.5	0.5

HECK APPROPRIATE BOX(ES)
(a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)
Evoked potentials at varying light intensities were examined in paranoid and nonparanoid groups of schizophrenics and in rapidly cycling bipolar affective disorder patients. Early components at 100-140 milliseconds differentiated paranoid from nonparanoids but did not change with mood cycles; late components did vary with affective mood.

Project Description:

Evoked potential research has been given emphasis by the unit because evoked potentials show high heritability and sensitivity to attentional as well as neuropharmacological manipulation -- coupled with the possibility of cortical or subcortical localization, these features make it an attractive technique for biological research in psychiatry.

Schizophrenic patients tend to have smaller and apparently more variable evoked potentials (EPs) than normal controls. The amplitude abnormality is consistent with a number of psychological theories of schizophrenia (e.g. Freud, Pavlov, Venables, Broen and Storms, Silverman) characterized by Epstein and Coleman as assuming that the "basic deficit in schizophrenia consists of a low threshold for disorganization under increasing stimulus input." While it is difficult to compare specific components in recordings made from different laboratories, there is general agreement that the P100-N140-P200 complex is smaller in schizophrenics, regardless of sensory modality. As predicted by the various theories, reduction in amplitude is particularly marked for high levels of stimulation such as rapid rates of stimulus presentation, high intensity, novel or paired stimuli.

Disorganized processing of sensory input in schizophrenics might also be apparent as their EPs seem to vary more from trial to trial. Also schizophrenics' responses to perceptual tasks seem to vary more from trial to trial than those of normals. Inability to maintain a stable response set has been postulated by Shakow to be a major psychological deficit in schizophrenics.

Techniques were developed for measurement of signal to noise ratio and response variability in single trial evoked potentials. These techniques were extended and verified using a digital computer simulation of signal plus noise trials. When applied to real data the measures appear to have high reliability and to demonstrate that human evoked potentials are more variable than would be expected from background noise variation alone. Empirical equations are presented which can be applied to existing single trial EP data to estimate both signal-to-noise ratio and its expected variance. These techniques were applied to 14 schizophrenic and 32 bipolar affective disorder patients, all tested off psychoactive medication 2 weeks or more.

For both visual and auditory stimuli, schizophrenics had lower SNRs. The ZA SNR measure on visual EPs differentiated the groups the most clearly (t of 4.24), showing an almost two-fold difference in mean SNR. Using the median SNR of the 72

normals and 14 schizophrenics (0.350) as dividing criterion would identify 13 of 14 schizophrenics but produce 34 false positive normals ($\chi 2$, $p<0.05$). The $\pm$ SNR measure also differentiated the schizophrenic and normal controls, although at slightly lower significance levels. Bipolar affective disorder patients, although having slightly lower SNR levels than normal controls showed no significant differences. SNR variability was also increased in the schizophrenic group, although this effect was only signigicant for tone stimuli. In all groups and for both modalities, the expected variance was signigicantly lower than the observed variance ($p<0.01$). Bipolar patients were also more variable in SNR from trial to trial than normal but significantly less variable than the schizophrenic group.

Efforts at developing a method of nonlinear transfer function analysis have resulted in a technique that is applicable to human EEG studies. A white noise stimulation experiment that allows identification of the Wiener kernels of a nonlinear system has been successful in modeling the human visual-EEG system. The resulting nonlinear transfer function will be useful in studying several aspects of EEG. Nonlinear interaction in the model predicts the facilitation effects that are seen in recovery cycle studies. Prediction of the transient EEG response agrees well with actual average evoked potentials to different intensities of visual stimulation. Other results suggest that the transient and steady state mechanisms of EEG response are more closely related than was previously believed. This type of comprehensive model will hopefully provide new ways to investigate evoked response mechanisms.

The Unit has continued to play a key role in developing the PDP/11 computer facilities for the NIMH Intramural Program. Richard Coppola has had an active part in setting up the new facility and in providing assistance to several of the projects utilizing the computer system. By insisting that investigators utilize a standard minimum hardware configuration, centralized development of general system software has aided greatly in getting laboratory computers up and running. The basic configuration is a PDP 11/10, DX11 dual flexible disk drive, AR11 analog subsystem and terminal, plus a custom standard interface panel for the AR11. Standard software running under RT11 then includes a display system, I/O command package, A/D and clock routines and other utility programs. A table driven experiment system was then written which allows fast setup of stimulus-response paradigms for a psychophysiology laboratory. Control of stimulus modality, intensity, duration, trial spacing, channel selection, sampling rate, averaging and artifact rejection is by a table of parameters interpreted by a

general experiment master program. The table is set up by an interactive program allowing easy experimental design. A flexible data base where format parameters are carried along with the data allows results from different experiments to be handled by one data management system. Automatic analysis programs then operate on this data base. The flexible disks then serve as the media for interaction with larger PDP 11/40 systems available for more advanced processing.

Publications:

Coppola, R., Tabor, R. and Buchsbaum, M. S.: Signal-to-noise ratio and response variability measurements in single-trial evoked potentials. Electroenceph. Clin. Neurophysiol., 44: 214-222, 1978.

Buchsbaum, M. S.: The average evoked response technique in the differentation of bipolar, unipolar and schizophrenic disorders. In Akiskal, H. (Ed.). Psychiatric Diagnosis: Exploration of Biological Criteria. New York, Spectrum, 1978.

Coppola, R.: A table driven system for stimulus-response experiments. Proceedings of the Digital Equipment Users Society, 3: 1219-1222, 1977.

Post, R. M., Stoddard, F. J., Gillin, J. C., Buchsbaum, M. S., Runkle, D. C., Black, K. E. and Bunney, W. E., Jr.: Alterations in motor activity, sleep and biochemistry in a cycling manic depressive patient. Archives of General Psychiatry, 34: 470-477, 1977.

Buchsbaum, M. S. and Coppola, R.: Signal-to-noise ratio and response variability in affective disorders and schizophrenia. In Begleiter, H. (Ed.). Evoked Brain Potentials and Behavior. New York, Plenum, (In press).

Coppola, R.: Computer applications in nonlinear modeling of EEG. Brain Theory Newsletter. (In press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00035-06 BP

PERIOD COVERED
October 1, 1977 through September 30, 1978

TITLE OF PROJECT (80 characters or less)

Biochemical and Psychopharmacological Correlates of the Average Evoked Response in Psychiatric Patients

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. S. Buchsbaum, M.D.	Chief, Unit on Percept. and Cognitive Studies	BPB NIMH
OTHER:	F. K. Goodwin, M.D.	Chief,	CPB NIMH
	D. L. Murphy, M.D.	Chief,	CNB NIMH
	R. D. Coursey, Ph.D.	Assoc. Professor, Dept. of Clin. Psychology	U. of Md.
	R. J. Haier, Ph.D.	Research Psychologist	LPP NIMH
	C. Schooler, Ph.D.	Research Psychologist	LSES NIMH
	T. P. Zahn, Ph.D.	Research Psychologist	LPP NIMH
	A. M. Ludwig, M.D.	Chairman, Dept, of Psychiatry	Univ.ofKy.

COOPERATING UNITS (if any) Clinical Psychobiology Branch; Clinical Neuropharmacology Branch; Laboratory of Psychology and Psychopathology; Laboratory of Socio-Environmental Studies; DCBR, NIMH. Dept. of Clinical Psychology, Univ. of Md. Dept. of Psychiatry, University of Ky.

LAB/BRANCH
Biological Psychiatry Branch

SECTION
Office of the Chief, Unit on Perceptual and Cognitive Studies

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.8	0.6	1.2

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A population of individuals potentially at risk for psychiatric disorders was identified by screening 375 college student volunteers for low platelet monoamine oxidase (MAO) activity Levels. The lower and upper 10 percent in MAO activity were interviewed and average evoked response, personality, perceptual and family history data obtained. Low MAO probands reported more frequent psychiatric or psychological counseling and problems with the law. Families of low MAO probands had an eight-fold increase in suicide incidence over those of high MAO probands. Both in these low MAO probands and in NIH psychiatric inpatients the combination of low MAO and average evoked potential augmenting was associated with increased frequency of suicide or suicide attempts. This suggests that reduced MAO levels, reported previously in patients with affective disorders and chronic schizophrenia, may predict a vulnerability to psychiatric disorder.

PHS-6040
(Rev. 10-76)

Project Description:

We previously found that the combined multivariate use of platelet MAO level and AER allowed us to successfully separate the four groups; normal, schizophrenic, bipolar, and unipolar affective illness, with 72% accuracy. However, since hospitalization stress, sensory isolation, dietary changes, etc., might affect both MAO and AER, we planned to locate normal individuals with extreme values of MAO and to study them with AER measures. This then represented a kind of biochemical "high-risk" study for the major psychoses.

The "high-risk" approach to the study of psychiatric disorders attempts to transcend the cause-effect dilemma by focusing on individuals believed vulnerable to such disorders before they become manifest. To select a pool of vulnerable individuals, nearly all high-risk studies have relied on genetic models and have examined the offspring or relatives of a clearly defined patient sample. Biochemical studies of psychiatric disorders, on the other hand, have usually begun with patients in "known" diagnostic groups and searched for abnormalities. The present study integrates the usual biochemical paradigm with the high-risk one to produce a new biochemically-at-risk strategy. Here, the "proband" is established by a biological measure, not a clinical one. The use of this strategy reduces the heterogeneity of the sample; eliminates the hospitalization, labeling and treatment factors that confound other biochemical studies; and rigorously tests the power of the biological factor under investigation.

Individuals potentially at risk for psychiatric disorder were identified by screening 375 college student volunteers for low platelet monoamine oxidase (MAO) activity levels. The lower and upper 10 percent in MAO activity were administered a personal and family history interview, psychological tests and average evoked response (AER) electroencephalographic procedures. Results indicated that low MAO males and females were socially more active, had more psychiatric contact, and had relatives who were psychiatrically more disturbed than high MAO subjects. Low MAO males had more convictions, experimented more with illegal drugs and had elevated scores on the MMPI. AER criteria further defined a high risk group of low MAO-AER augmenters which had more suicides among their relatives and higher scores on the schizophrenia scale of the MMPI.

A second study, utilizing a random sample of the 375 students replicates and extends earlier work by finding that low levels of platelet monoamine activity correlate with

stimulus-seeking, high ego strength, positive affect, and high activity levels, somewhat similar psychological correlates also being found for plasma amine oxidase activity. Although there are several ways in which a schizophrenia/MAO relationship may exist and still be congruent with the present data, these results pose difficulties for theories which link low MAO activity levels specifically to schizophrenia. Nothing in the present findings, however, is incongruent with the possibility of an association between low platelet MAO activity and bipolar affective disorder.

Chronic alcoholic patients were also studied, in collaboration with Dr. A. Ludwig, Chairman, Dept. of Psychiatry, University of Kentucky. Twelve alcoholic subjects were assigned to three different levels of audio-visual sensory stimulation (low, medium, high) and three different doses of alcohol (placebo, low, high) according to a balanced incomplete block design. The results indicated trend level and significant interactions between alcohol and sensory level effects on craving intensity and systolic blood pressure, respectively, as well as a number of other intriguing findings. Should the findings of this pilot study be replicated, they will have important implications for the understanding of alcoholism.

It is hypothesized that alcoholics possess a perceptual-neurophysiological sensitivity which renders them vulnerable to the effects of information or stimulus overload; the resort to alcohol, therefore, may represent an adaptive attempt to restore physiologic homeostasis through the pharmacologic "dampening" properties of this drug. Data on visual evoked potentials are currently being analyzed and will be reported next year.

In preparation for enlarged biological risk studies planned to exploit the biological high-risk strategy, we compared psychiatric evaluations made on the basis of psychometric screening with the MMPI to evaluations made on the basis of a standard clinical interview and the Research Diagnostic Criteria.

Initially, 385 college male volunteers completed the MMPI; two groups of individuals -- those with very high MMPI scores and those with scores within normal limits -- were selected for interview, evoked potentials and measurement of platelet monoamine oxidase. From the selected group, 15 met RDC criteria for major affective disorders and some correspondence between specific MMPI profile and RDC diagnoses was evident. The results indicated that we can identify a range of

psychopathology meeting RDC criteria by using the MMPI in a volunteer non-psychiatric population. This research sample, free from artifacts of hospitalization or drug treatment, can be especially useful in testing hypotheses concerning the biological correlates of psychopathology.

Publications:

Murphy, D. L. and Buchsbaum, M. S.: Neurotransmitter-related enzymes and psychiatric diagnostic entities. In Spitzer, R. and Klein, D. (Eds.): Current Issues in Psychiatric Diagnosis. New York, Raven Press, 1978, pp. 305-321.

Buchsbaum, M. S., Haier, R. J. and Murphy, D. L.: Suicide attempts, platelet monoamine oxidase and the average evoked response. Acta Psychiatrica Scandinavica, 56: 69-79, 1977.

Buchsbaum, M. S. and Murphy, D. L.: Peripheral monoamine enzyme indicators of psychological function and psychiatric susceptibilities. In Usdin, E., Wiener, N. and Youdim, M. B. H. (Eds.): Biochemistry and Function of Monoamine Enzymes New York, Dekker, 1977, pp. 941-962.

Ludwig, A. M., Wikler, A., Cain, R. B., Buchsbaum, M. S., Rinsky, G. and Taylor, R.: Alcoholics and high-low sensory input: A pilot study. Diseases of the Nervous System, 38: 681-683, 1977.

Buchsbaum, M. S., Murphy, D. L., Coursey, R. D., Lake, C. R., and Zeigler, M. G.: Platelet monoamine oxidase, plasma dopamine-beta-hydroxylase and attention in a "biochemical high risk" sample. J. Psychiatric Research (In press).

Coursey, R. D., Buchsbaum, M. S. and Murphy, D. L.: Platelet MAO activity and evoked potentials in the identification of subjects biologically at risk for psychiatric disorders. British J. Psychiatry, (In press).

Lipper, S., Murphy, D. L., Slater, S. and Buchsbaum, M. S.: Comparative behavioral effects of clorgyline and pargyline in man: A preliminary evaluation. Psychopharmacology, (In press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00036-04 BP

PERIOD COVERED
October 1, 1977 through September 30, 1978

TITLE OF PROJECT (80 characters or less)

Individual Differences in Sleep and the AER

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. S. Buchsbaum, M.D.	Chief, Unit on Percept. and Cognitive Studies	BPB NIMH
OTHER:	J. Leckman, M.D.	Resident, Department of Psychiatry	Yale Univ.
	J. C. Gillin, M.D.	Research Psychiatrist	BPB NIMH
	R. M. Post, M.D.	Sect. Chief, Section on Psychobiology	BPB NIMH

COOPERATING UNITS (if any)

Department of Psychiatry, Yale University

LAB/BRANCH
Biological Psychiatry Branch

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NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	0.2	0.3

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Earlier findings that 1) sleep deprivation might be beneficial in certain depressed patients and 2) that patterns of average evoked response (AER) were related to individual differences in sleep duration suggested the utility of the AER in clinical sleep deprivation studies. AER data were collected on normal controls, patients with affective illness and medication-free narcoleptics as a comparison. Primary affective disorder patients showed an increase in AER augmenting following sleep deprivation not shown by normal controls.

PHS-6040
(Rev. 10-76)

modulation of sleep, growth hormone and analgesia in normal man. In Jenkin, D. and Usdin, E. (Eds.): Cholinergic Mechanisms and Psychopharmacology. New York, Plenum Press (In press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z0 MH 00037-06 BP

PERIOD COVERED
October 1, 1977 through September 30, 1978

TITLE OF PROJECT (80 characters or less)

Genetic Factors in Psychiatric Illness and the AER

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. S. Buchsbaum, M.D.	Chief, Unit on Percept. and Cognitive Studies	BPB NIMH
OTHER:	E. S. Gershon, M.D.	Chief, Section on Psychogenetics	BPB NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH
Biological Psychiatry Branch

SECTION
Office of the Chief, Unit on Perceptual and Cognitive Studies

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.4	0.2	0.2

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Auditory and visual average evoked responses (AERs) were studied in families of patients with affective illness and in a small sample of twins with affective illness. In patients with primary affective illness, affective disorder probands and their siblings showed similar AER amplitude/intensity slopes, as did probands and their spouses. Within the probands' families, the "well" and "ill" relatives were not significantly different with both groups showing significant augmenting (high amplitude/intensity slopes) in comparison with normal groups. This suggests that AER stimulus intensity slope measures and affective disorders are not transmitted by a single genetic factor but that AER may be related to increased genetic vulnerability. Review of the heritability of other electrophysiologic variables also suggested that multiple loci and dominance, epistasis and interactions may be important.

PHS-6040
(Rev. 10-76)

Project Description:

The role of heredity in psychiatric illness is supported by the many studies demonstrating excessively high rates of illness in persons with a genetic relationship to known patients. The clinically variable expression of psychiatric disorders and the difficulty in identification of mildly affected relatives point to the need for biological markers for the genetic vulnerability. The heritability of AER measures combined with their applicability to both ill and well relatives makes them a promising approach. Further, differences have been demonstrated between patients with schizophrenia and various types of affective disorders.

Genetic mechanisms of the AER have been pursued in studies of pharmacological effects of amphetamine in normal volunteers. Four pairs of Mz twins have so far been tested on placebo controlled double-blind trials. Additional pairs will be tested during the next year.

Publications:

Buchsbaum, M. S. and Gershon, E. S.: Genetic factors in EEG, sleep and evoked potentials. In Davidson, J., Davidson, R. J. and Schwartz, G. E. (Eds.): Human Consciousness and Its Transformations. New York, Plenum Press (In press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z0 MH 00038-04 BP

PERIOD COVERED
October 1, 1977 through September 30, 1978

TITLE OF PROJECT (80 characters or less)

Laterality, Psychopathology and the AER

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. S. Buchsbaum, M.D.	Chief, Unit on Percept. and Cognitive Studies	BPB NIMH
OTHER:	D. P. van Kammen, M.D.	Unit Chief, Section on Neuropsychopharmacology	BPB NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH
Biological Psychiatry Branch

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INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.4	0.2	0.2

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Visual AERs from the left and right temperoparietal and occipital cortex were recorded using visual stimuli presented to the left and right hemiretinal fields and to both fields simultaneously. Subjects included normal controls and patients with acute schizophrenia, affective illness and right and left temporal lobectomies for epilepsy. One patient with partial agenesis of the corpus callosum was also studied. Differences in AER caused by attention were noticed especially in the left temperoparietal cortex when stimuli were presented separately to each field. Schizophrenic and lobectomy groups showed diminished asymmetry and attentional effects, whereas bipolar affective disorders showed generally enhanced attentional effects. Full visual field stimulation also produced attentional asymmetries, currently under study, in schizophrenics.

PHS-6040
(Rev. 10-76)

Project Description:

The left cerebral hemisphere has long been recognized as the major processing area for verbal information and the right hemisphere as the area for certain types of nonverbal spatial information. Recently, several psychophysiologic and neuroanatomic studies have suggested that a lateralized deficit may occur in schizophrenia. By presenting visual stimuli in the right and left visual field and recording EEG from the left and right hemisphere, AER techniques can take advantage of the anatomical arrangement of the human visual system to study abnormalities in lateralization. In normal subjects, AERs are highly asymmetrical and differ markedly with the task assigned the subject. AERs from the left temperoparietal area seem especially sensitive to changes in the attention given to the stimulus by the subject. Since attentional deficits have been one of the most widely postulated psychological deficits in schizophrenia, the application of this AER technique seemed promising. A group of 16 off-medication, acute schizophrenics and comparison groups of 17 affective disorder patients and 30 normal controls have been tested. As a further comparison, testing of 15 patients with right or left temporal lobectomies has been accomplished.

This technique required monitoring eye movements, restraining subjects' heads and other technical controls. In an effort to simplify the procedure, bilateral visual stimulation studies have been begun in schizophrenic, affective disorder patients and normals. Visual and auditory evoked potentials (EPs) were recorded from the right and left temperoparietal area in 30 right-handed adults under conditions of selective attention to the visual or auditory stimuli and during the execution of verbal and spatial tasks. Selectively attending to one modality over the other caused an increase in EP amplitude which was greatest for a negative component at 120-140 msec, and greater over the right than left hemisphere for both modalities. Nine patients given acute doses of amphetamine have also been tested and data is currently being analyzed.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00039-04 BP

PERIOD COVERED
October 1, 1977 through September 30, 1978

TITLE OF PROJECT (80 characters or less)

Sensory Thresholds and Averaged Evoked Responses

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. S. Buchsbaum, M.D.	Chief, Unit on Percept. and Cognitive Studies	BPB NIMH
OTHER:	W. E. Bunney, Jr., M.D.	Chief, Biological Psychiatry Branch	BPB NIMH
	G. C. Davis, M.D.	Sr. Staff, Unit on Studies of Drug Abuse	BPB NIMH
	F. K. Goodwin, M.D.	Chief, Clin. Psychobiology Branch	CPB NIMH
	R. M. Post, M.D.	Chief, Section on Psychobiology	BPB NIMH
	D. L. Murphy, M.D.	Chief, Clin. Neuropharmacology	CNB NIMH
	R. A. Lavine, Ph.D.	Dept. of Physiology	G.W.Univ.

COOPERATING UNITS (if any) Clinical Psychobiology Branch, DCBR, NIMH
Clinical Neuropharmacology Branch, DCBR, NIMH
Dept. of Physiology, George Washington University

LAB/BRANCH
Biological Psychiatry Branch

SECTION
Office of the Chief, Unit on Perceptual and Cognitive Studies

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.9	0.3	0.6

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Average evoked responses (AER) to varying intensities of electrical stimulation to the forearm and subjective ratings of these stimuli have been studied in 32 age and sex matched normal volunteers. Naloxone and aspirin and somatosensory AERs were also studied in normal volunteers.

PHS-6040
(Rev. 10-76)

(Continued)

OTHER: M. Webster — Research Assistant, Section on Psychiatry — LCS NIMH

Project Description:

In 1974 we found strong support for our concept of sensory augmenting/reducing. Individuals who were relatively pain tolerant as indicated by subjective shock ratings exhibited AER to electrical stimuli which decreased in amplitude with increasing stimulus intensity (reducing). A nonparametric signal detection analysis of subjective shock ratings has increased the precision of our AER-shock-rating relationships. This form of analysis is a powerful tool for analyzing pain experiments. It is currently being used in clinical studies in combination with AER techniques.

The discovery of opiate like peptides (endorphins) in brain raised questions as to their physiological role in pain perception and even led some to speculate on their role in affective regulation. Using somatosensory EPs and signal detection psychophysics, we were the first to report a hyperalgesic effect of the opiate blocker, naloxone, in man. We subsequently noted that the opposite of the specific EP augmenting effect caused by naloxone was produced by aspirin in a second controlled and double blind study. Bipolar and unipolar patients also differed strikingly on this component.

Data from other studies of effects of MAO inhibitors and cholinergic agents on evoked potentials is currently under analysis.

In the current year, we are further refining our EP/psychophysiological techniques with both methodological studies of EP component measurement and pharmacological ones including further examination of effects of naloxone.

Publications:

Buchsbaum, M. S., Davis, G. C. and Bunney, W. E., Jr.: Naloxone alteration of pain perception and somatosensory evoked potentials in normal subjects. Nature, 270: 620-622, 1977.

Buchsbaum, M. S. and Murphy, D. L.: Peripheral monoamine enzyme indicators of psychological function and psychiatric susceptibilities. In Usdin, E., Wiener, N. and Youdim, M. B. H. (Eds.): Biochemistry and Function of Monoamine Enzymes. New York, Dekker, 1977, pp 941-962.

Gillin, J. C., Sitaram, N., Mendelson, W. B., Buchsbaum, M. S., Moore, A. M., Jacobs, L. S. and Wyatt, R. J.: Cholinergic modulation of sleep, growth hormone and analgesia in normal man. In Jenkin, D. and Usdin, E. (Eds.): Cholinergic Mechanisms and Psychopharmacology. New York, Plenum Press, (In press).

Buchsbaum, M. S.: Neurophysiological studies of augmenting/reducing. In Petrie, A. (Ed.): Individual Differences in Pain and Suffering. Chicago, Univ. of Chicago Press, (In press).

Davis, G. C., Bunney, W. E., Jr., Buchsbaum, M. S., DeFraites, E. G., Duncan, W., Gillin, J. C., van Kammen, D. P., Kleinman, J., Murphy, D. L., Post, R. M., Reus, V. and Wyatt, R. J.: The use of narcotic antagonists to study the role of endorphins in normals and psychiatric patients. In Usdin, E. and Bunney, W. E., Jr. (Eds.): Endorphins and Mental Health Research. New York, MacMillan, (In press).

Haier, R. J., Rieder, R. O., Khouri, P. J. and Buchsbaum, M. S.: Extreme MMPI scores and the research diagnostic criteria: Screening males for psychopathology. Archives of General Psychiatry, (In press).

Lipper, S., Murphy, D. L., Slater, S. and Buchsbaum, M. S.: Comparative behavioral effects of clorgyline and pargyline in man: A preliminary evaluation. Psychopharmacology, (In press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00040-03 BP

PERIOD COVERED
October 1, 1977 through September 30, 1978

TITLE OF PROJECT (80 characters or less)

Amphetamine Pharmacokinetics and the Average Evoked Response in Hyperactive Children

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. S. Buchsbaum, M.D.	Chief, Unit on Percept. and Cognitive Studies BPB NIMH
OTHER:	M. H. Ebert, M.D.	Chief, Section on Exper. Therapeutics LCS NIMH
	G. L. Brown, M.D.	Medical Officer, Unit on Childhood Mental Illness BPB NIMH
	J. L. Rapoport, M.D.	Staff Psychiatrist, Unit on Childhood Mental Illness BPB NIMH

COOPERATING UNITS (if any)
Laboratory of Clinical Science, DCBR, NIMH

LAB/BRANCH
Biological Psychiatry Branch

SECTION
Office of the Chief, Unit on Perceptual and Cognitive Studies

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.2	0.1	0.1

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The effect of amphetamine on the average evoked response (AER) is being studied in hyperactive children. Blood amphetamine levels will be correlated with AER measures in an attempt to understand individual differences in response. The AER is only one phase of a larger project reported elsewhere. In an effort to demonstrate possible perceptual response stereotypy associated with amphetamine single evoked response trials are being collected for both visual and auditory stimuli, These single trials are then being analyzed for signal/noise ratio and trial-to-trial similarity using newly developed signal processing techniques.

PHS-6040
(Rev. 10-76)

Project No. Z01 MH 00040-03 BP

Project Description:

Previous research has demonstrated changes in average evoked responses (AER) with administration of amphetamine in children and in patients with affective disorders. Individual differences are prominent both in behavioral response as well as the AER; some individuals are aroused and others show sedation or no effect and AERs both predict and parallel these differences. We examined the behavioral, cognitive and EP effects of a single dose of amphetamine in 14 normal prepubertal boys in a double blind study. When amphetamine was given, the group showed a marked decrease in motor activity, improved vigilance performance and EP shifts away from augmenting. Very similar results, especially for EPs were obtained in hyperactive children. The similarity of the response to amphetamine in normal and hyperactive children cast doubt on the concept of a clinically specific or "paradoxical" response to stimulants in this syndrome.

During the next year, additional hyperactive children and normal adults will be studied to obtain age and dose-response curve data.

Publications:

Rapoport, J. L., Buchsbaum, M. S., Zahn, T. P., Weingartner, H., Ludlow, C. and Mikkelson, E. J.: Dextroamphetamine: Cognitive and behavioral effects in normal prepubertal boys. Science, 199: 560-563, 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00111-04 BP

PERIOD COVERED

October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Drug Abuse Studies/Stimulant Abuse Opiate Abuse

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.:

William E. Bunney, Jr., M.D.,	Chief	BPB,NIMH
Glenn C. Davis, M.D.,	Staff Psychiatrist, Unit on Drug Abuse	BPB,NIMH

Other:

Robert M. Post, M.D.,		BPB,NIMH
Michael Ebert, M.D.,	Chief, Section on Experimental Therapeutics	LCS,NIMH
Gerald L. Brown, M.D.	Medical Officer, Unit on Child Studies,	BPB,NIMH

COOPERATING UNITS (if any)

Laboratory of Clinical Science, NIMH

LAB/BRANCH

Biological Psychiatry Branch

SECTION

Unit on Studies of Drug Abuse

INSTITUTE AND LOCATION

NIMH, ADAMHA, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project is directed toward a basic understanding of the mood alterations induced by drugs of abuse. Several areas are under active investigation: the high produced by intravenous amphetamine and cocaine, chronic behavioral states caused by chronic use of stimulant drugs; neurological effects of chronic use of stimulant and opiate drugs.

Objectives

1. Drugs of abuse such as cocaine and amphetamine produce profound alterations in mood. There are major differences in acute and chronic administration. Our studies are designed to obtain a neurochemical understanding of these alterations.

2. Many individuals who use drugs chronically have motor and behavioral abnormalities. We are studying the causes and treatments for these abnormalities.

3. The chronic use of stimulant medications appears to produce depressions that mimic major depressive illness. Through study of the effects of the drugs, we hope to learn more about the etiology of affective illness.

Methods Employed

1. Patients who meet criteria for drug abuse stimulant-type, or drug abuse opiate-type are admitted to the Clinical Center Wards 2-West or 3-West for study and treatment.

(1.1) Treatment includes drug withdrawal and detoxification, if necessary, and careful medical evaluation for complications of chronic drug use (for example, hepatitis). Patients have the opportunity to become involved in intensive individual psychotherapy. Psychotherapy is directed toward individual needs and cognitive understanding of the function that drug use serves in the individual. Careful nursing intervention is offered as well, especially help directed towards dealing with common life-style problems manifested by a drug user in the ward situation.

(1.2) Historical and daily behavioral data. We obtain extensive histories of drug use including drug preference, frequency, chronicity and subjective effects of drugs. Other data are obtained in the ward situation utilizing nurse ratings of mood and behavior.

(1.3) Infusion studies. Many of the drug abuse studies center around the administration of various pharmacological agents. Amphetamine or cocaine are administered in non-tolerant patients (in doses that are less than the patient's previous habit). Behavior is rated by trained investigators along various dimensions, blood and urine are collected for amphetamine or cocaine levels, various catecholamines and their metabolites. Lithium or alpha-methyl-para-tyrosine (AMPT) is administered in placebo-controlled double-blind fashion to study the alterations of subjective effects produced by these compounds.

(1.4) Case reports. Patients suffering a variety of major side effects of drugs of abuse are admitted for exhaustive biochemical and behavioral studies.

Major Findings

1. Over ten patients have been admitted for studies with acute infusions of amphetamines or cocaine to date. Initial results indicate that these

patients meet most of the criteria for major affective illness. Amphetamine and cocaine injections under controlled conditions do not uniformly produce euphoria. In fact, the spectrum of mood effects of stimulants in these patients is wide. In the limited sample we have tested to date, neither AMPT nor lithium has produced consistent blockade of the mood effects. It is likely that these drugs have failed to block mood effects of stimulants because of the enormous variability of effects in chronic users.

2. Parkinsonism is a well recognized common side effect of drug treatment which either depletes central catecholamines or blocks dopamine receptors. Drug-induced parkinsonism usually develops over several weeks. When the offending drug is discontinued, remission is often gradual taking several weeks. This Unit has studied the acute development of parkinsonism in a young man subsequent to his parenteral use of a meperidine congener. This patient's symptoms persisted for eighteen months, but we have treated him successfully with bromocryptine, a potent dopamine receptor agonist.

3. In collaboration with the Unit on Childhood Mental Illness, we have studied the acute effects of methylphenidate in a young adolescent hyperactive child. This child abused methylphenidate. We found in controlled ward studies that he did not manifest typical euphoric or other subjective effects of high dose methylphenidate that is experienced by older drug abusers.

Significance to Biomedical Research

1. Drug abuse is a significant problem. A basic understanding of the mechanisms of action of drugs of abuse could lead to pharmacological agents useful in prevention, detoxification, withdrawal and long-term treatment.

2. The chronic use of stimulant and opiate medication produces clinical states similar to major affective illness. Thus, chronic drug use may provide a limited model of affective illness.

3. Motor side effects occur with most drugs of abuse. The linkage between motor effects and mood effects is an important one to the understanding both of affective illness and drug abuse.

Proposed Course

Studies are ongoing at this time.

Publications

1. Davis, G.C., Bunney, W.E., Jr., DeFraites, E.G., Kleinman, J.E., van Kammen, D.L., Post, R.M., and Wyatt, R.J.: Intravenous naloxone administration in schizophrenia and affective illness. Science 197: 74-77, 1977.

2. Goyer, P., Davis, G.C., and Rapoport, J.: Abuse of prescribed stimulant medication by 13 year old hyperactive child. J. Am. Acad. Child Psychiatry. In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00112-01 BP

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Endorphin Research in Mental Illness

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.:

William E. Bunney, Jr., M.D.,	Chief, Biological Psychiatry Branch	BPB,NIMH
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Other:

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Monte S. Buchsbaum, M.D.,	Unit Chief, Unit on Perceptual and Cognitive Studies	BPB,NIMH
Richard J. Wyatt, M.D.,	Director, Division of Special Mental Health Research	DSMR,NIMH

COOPERATING UNITS (if any)

Division of Special Mental Health Research, NIMH

LAB/BRANCH
Biological Psychiatry Branch

SECTION
Unit on Studies of Drug Abuse

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Endogenous opiate-like substances (endorphins) have recently been discovered. These substances function both as neurotransmitters and as neuromodulators within the central nervous system. Alterations in the function of endorphin mediated behaviors may play a role in mental illness. We are studying the action of endorphins on mood, memory, hormones, and learning. This Unit has tested the efficacy of narcotic antagonists in the treatment of affective illness and schizophrenia and will be studying the pharmacological and possibly therapeutic effects of beta-endorphin in man.

PrS-6040
(Rev. 10-76)

Objectives

In this project, we are seeking to understand the role of endorphins in normal physiology and in mental illness.

Methods Employed

Patients admitted on 2-West, 3-West, 4-East and at St. Elizabeth's are administered naloxone intravenously in double-blind placebo-controlled fashion to determine whether endorphins play a role in these illnesses. Clinical studies with the narcotic antagonist naltrexone are ongoing on 4-East. The intravenous administration of beta-endorphin to schizophrenic and depressed patients will begin shortly.

Major Findings

1. Six sleeping subjects were administered 2mg of naloxone, a pure narcotic antagonist in a placebo-controlled double-blind study. Naloxone failed to alter sleep variables, suggesting that endogenous opiate-like substances may not play a role in sleep regulation.

2. Three volunteers while participating in the sleep experiment had their respiratory rate measured during sleep by nasal thermister. These subjects received .4mg of naloxone 30 minutes after the onset of stage two sleep and then hourly for four hours. Respiratory rates determined for one minute intervals at every ten-minute segment of sleep were analyzed for REM and non-REM periods. Naloxone had no effect on respiratory rate during sleep.

3. Eight manic patients have been tested to date using doses ranging from .4mg to 30mg intravenously. One of eight patients receiving naloxone showed a dose-dependent improvement in the symptoms of "pressure of speech" and "pressure of thought." No other manic patient studied showed such an effect.

Significance to Biomedical Research

Endorphins might play an important physiological role in man. Results to date have failed to demonstrate an effect of naloxone on mood, respiration and sleep. Ongoing studies may unearth a role for endorphins in affective illness or schizophrenia.

Proposed Course

All studies are ongoing.

Publications

1. Davis, G.C., Duncan, W., Gillin, J.C., and Bunney, W.E., Jr.: Failure of naloxone to affect human sleep. Comm. Psychopharmacol. 1: 489-492, 1977.

2. Davis, G.C., Bunney, W.E., Jr., Buchsbaum, M.S., DeFraites, E.G., van Kammen, D.P., Kleinman, J., Murphy, D.L., Post, R.M., Reus, V.I., and Wyatt, R.J.: The use of narcotic antagonists to study the role of endorphins in normal and psychiatric patients. In Usdin, E., Bunney, W.E., Jr., Kline, N.S. (Eds.): Endorphins in Mental Health Research. New York, McMillan, in press.

3. Davis, G.C., and Bunney, W.E., Jr.: Introduction, review of clinical studies. In Usdin, E., Bunney, W.E., Jr., and Kline, N.S. (Eds.): Endorphins in Mental Health Research. New York, McMillan, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00113-01 BP

PERIOD COVERED

October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Psychophysiological Studies of Pain and Affective Illness

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.:

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Monte S. Buchsbaum, M.D.,	Chief, Unit on Perceptual and Cognitive Studies	BPB,NIMH

COOPERATING UNITS (if any)

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LAB/BRANCH

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Normal volunteers, affectively ill and schizophrenic patients participate in studies which use psychophysical and psychophysiological techniques to further our understanding of these illnesses and to determine the effects on perception of therapeutic drugs.

PHS-6040
(Rev. 10-76)

Objectives

1. To understand normal pain perception.

2. To understand how alterations in pain perception in affective illness and schizophrenia may give clues to other biological alterations in these illnesses.

3. To develop measures which will predict drug response among patient subgroups.

Methods Employed

1. Studies of affectively ill and schizophrenic patients hospitalized on the wards of the National Institute of Mental Health.

2. Normal volunteers are studied both in inpatient and outpatient experiments.

3. Procedures include visual, auditory, somatosensory evoked potential measurements; H-reflex; continuous performance measures; somatosensory stimulation with psychophysiological pain rating tests.

4. Double-blind, placebo-controlled drug administration.

5. Other psychosocial manipulations (e.g. attention).

Major Findings

1. Twenty-one normal adult volunteers participated in three similar evoked potential sessions. Each session consisted of two psychophysiological pain rating tests and a pain somatosensory evoked potential test. On two of the sessions the subjects received an intravenous injection of 2mg of naloxone or an equal volume of saline in a random order in double-blind fashion. Subjects were divided into pain sensitive and pain insensitive groups based on pain sensitivity level on familiarization day. Individuals defined as pain insensitive showed naloxone hyperalgesia, whereas pain sensitive individuals showed the opposite effect. Similar group differences were seen for evoked potential results. Our finding of naloxone effects on pain response supports the hypothesis that endogenous opiate-like substances have a physiological role in pain regulation.

2. A group of thirty-five normal subjects had somatosensory evoked potentials recorded and were tested on a psychophysical pain judgment task in the morning and afternoon sessions. Subjects tested in the morning were found to be significantly less pain sensitive than those tested in the afternoon on psychophysical judgments. E.P. measures were also consistent with pain insensitivity in the morning. In a second experiment, a group of twelve normal subjects also received naloxone in a double-blind trial, 6:00 in the morning and 6:00 in the evening. Naloxone hyperalgesia effects on the evoked potential were present in the morning and absent in the afternoon. Our results are consistent with the hypothesis that endorphins at least partially mediate diurnal variations in pain appreciation.

3. Complaint of pain is a prominent symptom in affective illness. Seventy-three affectively ill patients and forty-eight age and sex matched normal controls participated in a pain rating procedure consisting of single shocks administered at 2.5 second intervals. Male unipolar and bipolar depressed patients were significantly less pain sensitive than controls while female depressed patients were not. Bipolar depressed male patients demonstrated decreased sensory discrimination while unipolar patients only demonstrated a tendency to call higher intensity stimuli unpleasant. Both male and female manic patients were less pain sensitive across most measures.

Significance to Biomedical Research

1. The demonstration of pain tolerance to experimental stimuli among affectively ill patients suggests a possible link with endogenous opiate-like substances. Research in this area will contribute toward our understanding of affective illness and possible new therapeutic treatments.

2. Endorphins play an important physiological role in pain regulation in man. It appears that the diurnal release of endorphins may be linked to a physiological variation in pain sensitivity during the day.

Proposed Course

All studies are ongoing.

Publications

1. Buchsbaum, M.S., Davis, G.C., Bunney, W.E., Jr.: Naloxone alteration of pain perception and somatosensory evoked potentials in normal subjects. Nature 270: 620-622, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00070-05 BP

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Psychological and Biological Interactions in the Acute Psychoses

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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OTHER:	A. C. Rey, M.D.	Clinical Associate	BP NIMH
	J. C. Ballenger, M.D.	Clinical Associate	BP NIMH
	V. I. Reus, M.D.	Clinical Associate	BP NIMH
	E. K. Silberman, M.D.	Clinical Associate	BP NIMH
	W. G. Hamilton, M.D.	Clinical Associate	BP NIMH
	R. Savard, Ph.D.	Social Worker	BP NIMH
	A. Meyersburg, M.D.	Consultant	BP NIMH
	E. Silber, M.D.	Consultant	BP NIMH
	M. Skolnick, M.D.	Consultant	BP NIMH
	W. E. Bunney, Jr., M.D.	Collaborating Investigator, Chief, Biological Psychiatry Br.	BP NIMH

COOPERATING UNITS (if any)

Nursing Department, 3-West	NIH
Clinical Center Rehabilitation	NIH

LAB/BRANCH
Biological Psychiatry Branch

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Section on Psychobiology

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12.0	6.0	6.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project is involved in the detailed, longitudinal evaluation and treatment of patients with acute and chronic psychiatric illness, including depression, mania, schizoaffective illness, and drug-induced psychoses. The major research strategies include: precise longitudinal behavioral and biological measurements in the context of double-blind clinical trials of both routine and new, specific pharmacological agents. Neurotransmitter amines, metabolites, enzymes, electrolytes, cyclic nucleotides, polypeptide and related biological regulatory compounds are measured in body fluids of patients on and off medications and in different phases of illness and recovery. Neuroendocrine, motor activity, sleep, and averaged evoked responses are also monitored as well as specific measures of mood, cognition, abstraction, and verbal learning. An active animal and primate laboratory is maintained for the development of new techniques, including behavioral-biological studies not feasible in man, and studying animal "models" of psychosis, such as chronic cocaine-induced behavioral changes in the monkey and electrical kindling. An active interplay of psychological and biological perspectives thus occurs in seeking and developing new theories, research, and treatment approaches for a range of psychotic illnesses in man.

I. Project Description:

A. Objectives: The project is engaged in the multidisciplinary, longitudinal study and treatment of patients with a spectrum of acute psychoses, particularly involving mood disorders. Both investigative and treatment approaches focus on the elucidation of psychological and biological phenomena and their complex interactions.

B. Methods Employed:

1. Patients who meet formal research diagnostic criteria for manic-depressive illness, schizoaffective illness, schizophrenia, and drug-induced psychoses are admitted to the Section on Psychobiology, 3-West Clinical Research Unit. Normal volunteers are also admitted to provide control data for various psychological and biological projects.

2. During an initial drug-free interval, patients undergo extensive neurological, psychological, biochemical, and neurophysiological evaluation. Treatment and clinical evaluation are begun in individual and group psychotherapy as well as ongoing clinical case conferences. Behavioral data are collected twice daily, both by a trained nursing team and by patient self-ratings. Nurses also evaluate patients on a modified Brief Psychiatric Rating Scale (BPRS) three times weekly. Motor activity is assessed in collaboration with Dr. Theodore Colburn utilizing a miniaturized activity monitor worn on the non-dominant wrist.

3. Clinical and neuroendocrine parameters are evaluated during testing with apomorphine, L-DOPA, amphetamine, and other agents such as thyrotropin releasing hormone.

4. Amine metabolites and electrolytes are systematically evaluated utilizing urinary, blood, and cerebrospinal fluid approaches to neurotransmitter metabolism.

5. Both routine and experimental compounds are evaluated during double-blind clinical trials. Routinely used drugs are tricyclic antidepressants, lithium carbonate, monoamine oxidase inhibitors, and neuroleptics. Experimental drugs include the direct dopamine agonist piribedil, the anticonvulsant carbamazepine, the narcotic antagonist naloxone, and the endogenous polypeptides adrenocorticotropic releasing factor (ACTH) and β-endorphin.

6. The transient antidepressant effects of one night's sleep deprivation in patients with severe depressive mood disorder is examined and underlying neurobiological and neuroendocrine changes examined.

7. Animal Models. A rodent and primate behavioral pharmacology laboratory is maintained in order to develop new research techniques in the longitudinal study of biobehavioral interactions. Particular focus is given to the study of chronic cocaine-induced behavioral pathology and the underlying changes produced by repetitive stimulation of the amygdala (electrical kindling).

C. Major Findings:

1. A New Treatment Strategy for Manic-Depressive Illness: Use of the Temporal Lobe Anticonvulsant Agent Carbamazepine (Tegretol). A detailed series of clinical and biological studies of carbamazepine have been undertaken in the Section on Psychobiology in collaboration with James Ballenger and William E. Bunney, Jr. The clinical trial of carbamazepine was undertaken in light of the consistent positive effects on psychiatric symptoms in patients with psychomotor epilepsy, the predominantly temporal lobe and limbic effects of the drug, its ability to inhibit limbic seizures, kindling, and other model psychoses, and preliminary data in open trials in Japan that the drug might have some efficacy in manic-depressive patients. Initial data in our double-blind, clinical evaluation indicate that the drug has marked antimanic effects in some patients. To date, five of seven patients have shown evidence of an antimanic response, with three showing both dramatic improvement on the drug and relapse upon placebo substitution. These positive responses have been observed in some patients who have been previously noted to be lithium nonresponders. In addition to acute antimanic effects, several patients have been maintained on carbamazepine for longer periods of time with evidence of sustained or prophylactic effects against their recurrent mood disorder. Our preliminary data also suggest that carbamazepine may have some acute and prophylactic effects in the depressed phase of illness; three of eight patients have responded to date. One of these patients has shown evidence of a sustained prophylactic response.

Further work needs to be done in patients with psychiatric illness to determine whether the predominantly limbic and temporal lobe anticonvulsive effects of carbamazepine (in comparison to the predominantly cortical effects of diphenylhydantoin) are related to its psychotropic effects in manic-depressive illness. Biochemical studies indicate that carbamazepine may affect a variety of neurotransmitter systems. Patients did not show a consistent alteration in the urinary norepinephrine metabolite MHPG during carbamazepine treatment. However, cerebrospinal fluid norepinephrine measured in collaboration with Ray Lake, decreased in manic patients treated with carbamazepine, but showed little change or increased in depressed patients treated with the drug. These preliminary findings would be consistent with biochemical studies indicating that the drug not only blocks reuptake like other tricyclic antidepressants, but may block stimulated induced release of norepinephrine as well. Cerebrospinal fluid accumulations of homovanillic acid, the dopamine metabolite, were significantly decreased in patients treated with carbamazepine. The possible effects of carbamazepine on dopaminergic activity in relation to the antimanic and antidepressive effects of the drug remain to be elucidated.

If the preliminary evidence of carbamazepine's acute and prophylactic effects in manic and depressive illness continue to be substantiated in further work, these data would suggest that it could play a unique role in affective illness. It is useful in some patients who are lithium nonresponsive and may be of use in those unable to take lithium carbonate because of side effects. It may also be of use in patients who require neuroleptic treatment in addition to lithium carbonate for management of recurrent manic or schizoaffective mood disturbances. In the wide experience with the drug in patients with epilepsy, there has been no reported incidence of tardive dyskinesia which is common

with chronic neuroleptic treatment. In addition, because of its spectrum of therapeutic effects in patients with psychomotor epilepsy and in patients with affective illness, the drug may provide new clinical and theoretical leads to the interrelationships between these syndromes and their possible underlying neurochemical and biological substrates.

2. The Involvement of Dopamine in Mania and Depression: Implications for Treatment.

a. Piribedil is a drug which acts by directly stimulating dopamine receptors in a relatively specific fashion. As such, it is an important clinical treatment strategy as well as a research tool in dissecting the possible role of dopaminergic mechanisms in affective illness. Our clinical evaluation of piribedil continues to suggest that some depressed patients show substantial clinical improvement on the drug while others show only mild to moderate changes. One depressed patient became worse and one showed the emergence of manic symptomatology during treatment of his depression. Our preliminary data indicated that this range of antidepressant responsivity was related to pretreatment levels of dopaminergic activity as measured with the CSF techniques. Whether or not this biological predictor continues to be a reliable indicator of piribedil response in a larger series of patients, the range of drug responsivity does suggest that different patients may be differentially sensitive to a relatively specific manipulation of their dopaminergic system. Several of the most dramatic responders to piribedil have been previously nonresponders to routinely used tricyclic antidepressant compounds which are thought to affect predominantly serotonin and norepinephrine mechanisms. These data may indicate a useful role of a dopamine active agent in a specific subgroup of depressed patients.

b. Low doses of piribedil like those of other direct dopamine receptor agonists may act preferentially on presynaptic dopamine receptors and produce inhibitory effects on behavior. A wider clinical trial of this low dose dopamine agonist strategy in mania is currently in progress.

c. Pimozide is an antipsychotic drug which has more specific effects in blocking the dopamine receptor than the other commonly used agents. Eight of nine manic patients studied in collaboration with Drs. Goodwin, Bunney, and Jimerson have shown notable antimanic responses to this compound. These data again are highly suggestive of a role for dopaminergic hyperactivity in mania and indicate that blockade of dopamine receptors is associated with therapeutic effects in manic patients.

3. Lithium Carbonate Distribution and Mechanism of Action.

a. Studies in collaboration with Alix Rey and David Jimerson indicate that cerebrospinal fluid levels of lithium measured after seven to ten days of treatment are substantially lower than those measured after three weeks. This lag in achieving adequate lithium levels as measured in the cerebrospinal fluid with a routine pattern of lithium administration suggests that it may be one factor in the slow onset of lithium's therapeutic effects in manic and depressed patients. Although a wide range of cerebrospinal fluid levels were observed during acute and chronic lithium administration, within the "therapeutic range" as judged by serum levels, the CSF levels did not relate directly

to degree of clinical response, however.

b. Acute lithium administration was associated with decreases in cerebrospinal fluid levels of calcium and magnesium. Lithium's effects on these divalent cations may be linked to its therapeutic effects in the illness, although considerable further investigation of this hypothesis is required.

c. In the animal laboratory, chronic lithium administration is not associated with blockade of psychomotor stimulant effects. However, following lithium withdrawal, animals are less reactive to psychomotor stimulant administration. These data may have implications for the time course of emergence of clinical relapse during the lithium withdrawal period.

d. In collaboration with Victor Reus and Herbert Weingartner, a subtle pattern of memory impairment during chronic lithium administration has been observed. Bipolar manic-depressive patients maintained on lithium had a deficit in the consistency of the recall of word lists in comparison to bipolar patients withdrawn from lithium administration. Further clarification of lithium induced side effects on memory and cognition may be of particular clinical importance in evaluating side effects and in dealing with issues of drug compliance.

e. In collaboration with Elliot Gershon, John Nurnberger, Monte Buchsbaum, and Dennis Murphy, several biological predictors of the degree of antidepressant response to lithium have been documented. Depressed patients showing the most augmenting response on average evoked potential testing during a medication-free interval prior to lithium administration and those patients with the highest levels of platelet monoamine oxidase activity had the best antidepressant response to lithium carbonate.

4. Amphetamine: The Role of Catecholamine Activation in Depression. Clinical, biological, and cognitive responses to amphetamine have been studied in more than 20 depressed patients in collaboration with David Jimerson, Victor Reus, Edward Silberman, and William E. Bunney, Jr. Approximately half of the depressed patients studied following intravenous amphetamine (20 mg) during a double-blind diagnostic clinical interview showed mood elevation and approximately one half showed prominent affective arousal and catharsis. These two aspects of the amphetamine response are independent, however, and indicate the complexity of the interaction of amphetamine with the underlying clinical and biological state. The degree of amphetamine induced mood change and catharsis appears to be a useful clinical indicator of the kind of response observed during psychotherapy, however. Previous data suggested that the degree of serotonergic tone was inversely related to the presence of amphetamine induced dysphoria. Patients with a reducing pattern on average evoked response showed a euphoric response, however (in collaboration with Monte Buchsbaum).

In collaboration with Drs. Reus and Weingartner, an effect of amphetamine on memory and learning was observed. Patients showed improvement on free but not cued recall of word lists during amphetamine administration compared to a placebo. The effect was increased in more deeply processed information. These data are compatible with studies in other patients including normal and hyperactive children and normal volunteers indicating that amphetamine increases

free recall of more deeply encoded words.

In addition, in patients with higher pretreatment levels of catecholamine metabolites in the cerebrospinal fluid amphetamine produced subjective and objective evidence of greater cognitive facilitation than those with lower pretreatment levels. These data indicate that in patients with low noradrenergic tone, amphetamine will enhance cognitive functioning, but in patients with already high noradrenergic function, amphetamine may produce negative effects. Again, these data suggest that baseline differences in clinical and biological functioning interact importantly with pharmacological response.

5. Therapeutic Effects of Sleep Deprivation in Depressed Patients. We continue to observe transient clinical improvement following one night's sleep deprivation in depressed patients compared to normals. These studies implicate alterations in circadian rhythms in the mood altering effects. Depressed patients who responded positively to sleep deprivation had normal diurnal variation in temperature while nonresponders had flat temperature curves. As previously described, normal volunteers did not respond with mood elevation to the sleep deprivation procedure but complained of mild increases in fatigue, irritability, and dysphoria in contrast to the depressed patients. Clinical improvement of the depressed patients interacted with CSF calcium and MHPG levels, changes in the average evoked response, morning plasma cortisol levels, and serum calcium changes. Further investigation of the paradoxical antidepressant response in depressed patients compared to normal controls following one night's sleep deprivation should provide important clinical and biological clues to the nature of depressive process. In Europe, repeated deprivation procedures are used as clinically effective antidepressant strategies in combination with the tricyclic antidepressants.

6. Biological Rhythms and Mechanisms in Rapidly Cycling Manic-Depressive Patients.

a. Melatonin Rhythms. Following initial studies in collaboration with Drs. Jimerson, Lynch, Wurtman, and Bunney, diurnal variations in urinary and plasma melatonin excretion have been studied in depressed patients and volunteers. One night's sleep deprivation did not change the pattern of urinary excretion. However, in four normal volunteers who underwent a ten day phase reversal study (during which time they slept during the day and were awake during the night), a shift in urinary melatonin was observed. Four to five days after the phase shift, urinary and plasma melatonin excretion altered its circadian pattern and peak levels were observed during the time the volunteers were asleep.

b. In rapidly cycling manic-depressive patients, we have continued to observe significant alterations in a variety of biological parameters. CSF norepinephrine and urinary MHPG excretion are lower in the depressed compared with the manic phase. At the same time, urinary free cortisol (studied in collaboration with Drs. Ballenger and Gold) is significantly lower in the manic phase than the depressed phase. CSF calcium is lower during mania than depression. Although activity, stress, anxiety, and other potentially confourding factors remain to be ruled out, these data support a role for altered noradrenergic, endocrine, and electrolyte function in manic compared to depressed phases of the illness.

CSF levels of the inhibitory neurotransmitter GABA have been measured in the CSF. In one rapidly cycling patient, values were significantly higher in mania than in depression. These preliminary data mitigate against the postulated GABA deficit which would secondarily increase dopaminergic activity in mania.

7. Cognitive Functions in Affective Illness.

a. Hemispheral Laterality. A variety of clinical data implicate alterations in the balance in left or right hemispheral function in mood and cognitive disturbances in affective illness and schizophrenia. Preliminary data in collaboration with Victor Reus, Monte Buchsbaum, and Herbert Weingartner indicate that amphetamine produces selective hemispheral activation in association with mood elevation in depressed patients.

b. State-Dependency. A wide variety of studies in the state-dependent learning literature have been reviewed and integrated in collaboration with Victor Reus and Herbert Weingartner. These studies suggest that not only are state-dependent alterations in learning and memory observed following pharmacological manipulations but also following endogenous chemical changes and spontaneous or experimentally induced alterations in mood. These data have important implications for the accessibility of information across mood states in affectively ill patients. They also suggest a specific set of psychotherapeutic strategies geared toward overcoming state-dependent cognitions in order to facilitate psychotherapy and the rapidity of clinical improvement.

c. Marked cognitive and thinking pattern disturbances have been observed in depressed patients (Silber, Rey, and Savard). The depressed patients demonstrate cognitive rigidity and, paradoxically, an inability to express depressive affect. This affective inaccessibility, rather than an excess of depressive affect, may be an integral part of the depressive process. The degree of depressive thinking disturbance was significantly related to a reducing pattern on the average evoked potential response, indicating a possible association between electrophysiological and clinical measures of cognitive processing.

d. Depressed patients, compared to carefully matched control populations, display marked defects on the Halstead Category test. The defect is particularly marked in bipolar patients, especially those in the older age ranges. This deficit is particularly important since it appears specific to several of the subtests of this procedure and does not represent a generalized defect which could otherwise be attributable to anxiety, lack of attention, or some other secondary variable. It appears that depressed patients have specific and selective impairments, possibly related to left hemispheral function.

e. In collaboration with Dr. Meyersburg, a manuscript entitled "An holistic developmental view of neural and psychological processes" has been prepared for publication. It suggests earlier psychoanalytic concepts may be usefully re-explored in light of newer neurobiological findings.

8. Kindling as an Etiological Mechanism in the Alcohol Withdrawal Syndromes (in collaboration with James Ballenger). Data collected at the Bethesda Naval Hospital indicate an increased severity of alcohol withdrawal symptoms in relation to duration of alcohol abuse. Dr. Ballenger has integrated

these data around the kindling hypothesis, suggesting that each episode of alcohol withdrawal (which is associated with hyperexcitability of a variety of brain substrates) acts as a kindling stimulus and is associated with increasingly severe reactions upon sequential withdrawals. Implicit in this interpretation of the data is the notion that the increased duration of alcohol abuse is associated with a large number of withdrawal episodes. Even continuously drinking alcoholics appear to go through episodes of partial withdrawal following each night of sleep and alcohol abstinence. More major episodes of alcohol withdrawal may occur around intercurrent medical problems, voluntary abstinence, or hospitalization. Considerable data in the animal literature is consistent with a notion that each episode of alcohol withdrawal is associated with increasingly severe symptomatology. If this model has general validity, not only would it represent a new conceptual approach to alcohol withdrawal symptomatology and the personality changes associated with the syndrome, but it might also lead to new treatment strategies aimed at blocking the recurrence of the withdrawal syndrome. It is also consistent with the notion that chronic alcoholism and episodes of withdrawal are capable of producing long lasting changes in biological and behavioral responsivity.

9. Cerebrospinal Fluid Studies as an Approach to Central Neurotransmitter Metabolism (in collaboration with Frederick Goodwin, James Ballenger, Edna Gordon, and William E. Bunney, Jr.).

a. Normal Volunteers. An extensive study of central neurotransmitter and neuromodulatory substances is being conducted in normal volunteers. This data will provide important control group values in affectively ill and psychotic patients. In addition, extensive psychological testing will allow evaluation of possible relationships between central neurotransmitter function and behavior in the normal range.

b. CSF Norepinephrine. In collaboration with Dr. Ray Lake, CSF norepinephrine continues to be measured utilizing a sensitive radioenzymatic assay in patients with manic and depressive illness and in normal volunteers. It was found that manic patients have significantly higher baseline and probenecid-induced accumulations of norepinephrine than do depressed patients, neurological controls, or a recently studied group of normal volunteers. Increased psychomotor activity accompanying mania may be a partial explanation for these findings. However, some manic patients who were maintained on strict bedrest had markedly elevated CSF norepinephrine and the relationship of these elevated levels to the manic syndrome remains a promising lead for further exploration. Values of norepinephrine in depression were not significantly different from those in neurological controls. Patients with high anxiety did have significantly higher levels of norepinephrine compared to depressed patients with low anxiety, however. Since CSF norepinephrine does not appear to be derived from peripheral metabolism, these data represent one of the first direct tests of the catecholamine hypothesis, possibly implicating elevated levels of norepinephrine in mania. Depressed patients with lower norepinephrine had less functional deficit and more improvement in free recall following amphetamine than did patients with higher norepinephrine.

c. Cerebrospinal Fluid Dopamine Metabolites. While depressed patients do not appear to have significantly different levels of the dopamine

metabolite homovanillic acid (HVA) in cerebrospinal fluid compared to other psychiatric comparison populations, the range of HVA values may be clinically relevant. Preliminary studies suggest that patients with evidence of low HVA accumulations following probenecid administration (a measure of dopamine turnover) show best response to treatment with a dopamine receptor agonist such as piribedil. Similarly, patients with low baseline HVA levels responded with the greatest antidepressant effects to the sleep deprivation paradigm. Thus, patients with evidence of low central dopamine function may be differentially responsive to clinical antidepressant strategies even though the overall HVA in depressed patients is not significantly different from controls.

A methodological study has been completed indicating a high reliability seen in HVA measured by mass fragmentographic and earlier fluorometric techniques. The mass fragmentographic assay is clearly the more precise and reliable, however.

d. CSF Serotonin Metabolism and Subgroups of Patients with Affective Illness. In collaboration with Frederick Goodwin, preliminary data suggest that patients with low serotonin turnover, as estimated by accumulation of 5-hydroxyindoleacetic acid (5HIAA) following probenecid, may have higher urinary MHPG as an index of noradrenergic function. These differential neurotransmitter alterations may differentially relate to subsequent pharmacological response.

e. Thus, the preliminary data available for serotonin, norepinephrine, and dopamine metabolites suggest that depressed patients with different biological abnormalities may be differentially responsive to pharmacotherapy and that establishing a given patient's biological profile, which may be independent of clinical phenomenology, may ultimately have important clinical and therapeutic implications.

f. CSF Calcium. Work initiated in collaboration with John Carman and David Jimerson has been continued with James Ballenger and William Hamilton. Baseline levels of CSF calcium tend to be lower in normal volunteers than in the depressed population as a group and bipolar depressed patients in particular. There is a significant positive correlation of severity of depression and CSF calcium measured in either baseline 9:00 a.m. samples or at 3:00 p.m. following probenecid administration. These correlational data suggesting that more severely depressed patients have higher CSF calcium are consistent with paired data in individual patients demonstrating rapid mood cycles. In these patients, CSF calcium levels are significantly higher during the depressed phases of illness than during the manic phases.

g. A variety of treatment strategies also appeared to decrease CSF calcium in association with antidepressant response. As previously reported, successful treatment of severely depressed patients with electroconvulsive therapy is associated with a significant decrease in CSF calcium. The degree of improvement in depression during lithium therapy was correlated with the degree of decrease in CSF calcium. There also was an interaction of sleep deprivation response with CSF calcium change--those improving had decreases in CSF calcium while those who became worse had increases in calcium. A larger group of depressed patients has now been restudied during a medication-free interval, and

it continues to show decreases in CSF calcium compared to levels when patients were depressed. As previously noted (in collaboration with Daniel van Kammen), recovery from an acute schizophrenic episode was associated with a significant increase in CSF calcium. CSF calcium in medicated patients with seizure disorders studied in collaboration with James Wood tended to be lower than neurological controls. These data on CSF calcium in a variety of clinical states continue to be consistent with the experimental literature suggesting that decreases in CSF calcium are associated with increased neuronal irritability while increases in CSF calcium tend to act as an anticonvulsant and decrease behavioral arousal. Several different clinical approaches suggest an association between CSF calcium and mood disturbances--higher levels of calcium with more severe depression and decreased levels following clinical improvement with a variety of strategies.

h. Dopamine β-hydroxylase (DBH) (in collaboration with Pauline Lerner). There were no significant differences between cerebrospinal fluid DBH in depressed patients and normal volunteers. In contrast to the higher levels of norepinephrine observed during mania, DBH levels were lower in mania. However,, levels of DBH in depressed patients were significantly correlated with levels of norepinephrine in CSF suggesting that, at least in the depressed state, DBH may be released into CSF in close association with its neurotransmitter end product. DBH may provide an important central enzyme marker of noradrenergic activity in some instances.

10. The Polypeptide Neuromodulators ACTH and β-endorphin.

a. Adrenocorticotropic hormone (ACTH) and an analogue (Organon 2677) with central effects but which does not stimulate the adrenals have been given to depressed patients (by Dr. Reus). Continuous performance tasks showed improvement compared to placebo, and alterations in mood, cognition, and biochemistry are being studied. A large body of data suggests that there are prominent alterations in the pituitary adrenal axis during depression.

b. Naloxone. If the endogenous opiate β-endorphin is involved in mood and cognitive disturbances in the psychoses as has been postulated, then block of opiate receptors by naloxone (a specific opiate antagonist) should affect these illnesses. In collaboration with Drs. Davis, Reus, and Bunney, naloxone has not generally reversed manic behavior, although reliable decreases in selective symptoms have been observed in one manic patient. Direct studies of β-endorphin in affective illness are being initiated.

11. Models for the Development of Psychosis.

a. In many studies we have documented that repeated administration of the psychomotor stimulant cocaine produces increasing behavioral pathology in the rat and rhesus monkey. The underlying biochemical and electrophysiological changes associated with the phenomenon are being studied.

b. Kindling. Repeated cocaine and lidocaine administration also lead to the development of seizures in a pattern that resembles electrical kindling. This increased susceptibility to convulsive activity led us to study

the interaction of pharmacological sensitization phenomena with the electrical kindling paradigm (with Kathleen Squillace and Agu Pert). Kindling involves daily electrical stimulation of various limbic sites, particularly the amygdala, with current which is initially subthreshold for EEG or behavioral effects. Repetition of this stimulation (200 microamphs, 50 Hz/500 milliseconds) eventually is associated with increasingly long amygdala after-discharges and finally culminates in the animal having a major convulsive seizure.

c. Amygdala kindling in this fashions alters behavioral responsivity to several drug challenges. Kindled animals compared to implanted sham-stimulated controls have significantly decreased cocaine-induced vertical hyperactivity; they also have a significantly higher incidence of lidocaine-induced convulsions than do nonkindled controls. These data suggest that relatively long-term alterations in behavioral responsivity and in convulsive threshold may be achieved with electrical kindling. This procedure may represent a useful strategy for producing long-term alterations in excitability of specific substrates important in the regulation of emotional and cognitive behavior.

d. In three separate amygdala kindling studies, approximately 40% of the animals developed marked oscillations in after-discharge duration. Some animals showed a tendency to 48-hour cycles, i.e., they demonstrated long after-discharges lasting approximately two minutes on one day and short after-discharges of ten to thirty seconds on the next, and so on. These differences in after-discharge duration were also associated with the appearance of major seizures on the days when long after-discharges were present. It is suggested that these marked oscillations in after-discharge duration reflect basic rhythmic alterations in amygdala excitability which may be of behavioral consequence and may be a useful model for other central nervous system cyclic phenomena. Preliminary studies suggest that lithium did not alter the incidence of development of the oscillatory pattern, however.

e. Study of the recurrent motor activation in animals with stimulants may be a useful model for mania and the tendency for rapid cycling. The data implicate alterations in dopamine receptor function and have led to the use of the anticonvulsant carbamazepine in affective illness.

D. Proposed Course of the Project.

1. Studies of the mechanism of action on a biochemical and endocrinological level of carbamazepine in affectively ill patients will be continued. We will also seek clinical and biological profiles for which patients might respond best to the drug since it appears clinically effective in some lithium nonresponders.

2. Alterations in receptor mechanisms in affective illness will be studied using the dopamine receptor agonist piribedil and antagonist pimozide.

3. A life chart approach will be used for mapping the course of affective episode and therapeutic interactions. In this fashion, the role of life events in precipitating the illness can be examined.

4. Studies of polypeptide neuromodulators such as β-endorphin, ACTH,

and their analogues will continue.

5. A variety of techniques for assessing CNS function using the neuroendocrine approach are being investigated. These include administration of thyrotropin releasing hormone (TRH), luteinizing releasing hormone (LHRH), arginine, apomorphine, and hypertonic saline. Alterations in target substances such as TRH, LH, growth hormone, prolactin, and vasopressin in blood and CSF will be examined in affectively ill patients before and during pharmacological treatment.

6. Detailed exploration of cognitive alterations in affective and schizoaffective illness will continue on several levels including the examination of the role of hemispheral laterality.

7. Elucidation of biochemical correlates of normal behavior will continue centered around establishing clinical and biological profiles in normal volunteers.

8. Use of spinal fluid techniques in man as an approach to central nervous system neurotransmitter and modulatory functions in the ill and well state will continue.

9. Clinical and laboratory studies of long-lasting behavioral and biological changes induced by chronic drug or physiological (kindling) treatments will be extended.

10. The relevance of seizure disorders and laboratory seizure models to psychiatric psychopathology and its underlying anatomical, physiological, and biochemical substrates will be explored..

11. Thus, the work on the unit will continue to attempt to integrate multiple psychological and biological perspectives in the study and treatment of patients with a spectrum of severe psychiatric disorders, especially the mood disorders. Basic to this approach is the separation of genetic, biological, and experiential predispositions from state-related biological changes in the illness. As in the animal models discussed, particular attention will be paid to longitudinal changes in both the clinical phenomenology and in underlying biochemical changes since much evidence suggests that alterations during an acute episode are very different from those occurring later in the illness.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 00049-04 BP

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Psychosocial Factors in the Prognosis of Melanoma

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Daniel P. van Kammen, M.D.	Unit Chief, Section on Neuropsychopharmacology	BPB	NIMH
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J. Rosenblatt	Clin. Assoc., Sec. on Neuropsychopharmacology	BPB	NIMH
D. Sternberg	Clin. Assoc., Sec. on Neuropsychopharmacology	BPB	NIMH

COOPERATING UNITS (if any)

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.6	0.5	0.1

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

We are studying the effect of various psychosocial factors on host resistance to malignant melanoma. A detailed characterization of these factors in patients with melanoma, Clinical Stage II, is being obtained. In addition certain endocrine and immune parameters are also being studied. We are testing several hypotheses which may distinguish those patients suffering recurrence of their disease from others who either remain disease-free or have a delayed recurrence. At one year, significant correlations with disease-free status were found with (1) number of positive lymph nodes, (2) ABO type, (3) social assets, and, (4) melanoma adjustment self-rating score.

In a second group of 30 melanoma patients, findings (1) and (4) were replicated as predictors of relapse within one year.

PHS-6040
(Rev. 10-76)

Objectives

This study is aimed at developing a detailed characterization of the psychosocial factors which distinguish those patients who suffer recurrence of malignancy from those who remain disease-free or who have a delayed recurrence. Data is being gathered to test several current hypotheses concerning psychosocial antecedents of the onset of physical illness and predisposing psychobiological states.

Fifty to 100 patients with "surgically-cured" Stage II Melanoma will be studied. We plan a multi-level assessment of each patient. In addition to the biological measures gathered on the patient, we will characterize the patient's genetic history, past psychosocial history, level of personality development, current life stress and present psychological state. We will accomplish this by (1) psychiatric interview, (2) objective assessment of a five-minute speech sample, and, (3) standardized questionnaires including MMPI, Holmes and Rahe Schedule of Recent Experience, SCL-90, Rotter Internal-External Locus of Control Scale, Beck Depression Inventory and the Gottschalk In-Out Scale.

The patients are seen shortly after their "curative" surgery and will be followed up at 2-month intervals, at which time we will obtain the Holmes-Rahe Scale, the SCL-90, Helplessness-Hopelessness Index and Beck Depression Inventory.

At one year, significant correlations with disease-free status were found with (1) number of positive lymph nodes, (2) ABO type, (3) social assets, and (4) melanoma adjustment self-rating score. We are presently examining this finding in a second group of patients. A second paper was presented at the American Psychosomatic Society meeting.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00132-04 BP

PERIOD COVERED

October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Biological and psychopharmacological evaluation of schizophrenia

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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Clinical Branch, NEI; Immunology Branch, NCI; Laboratory of Clinical Psychopharmacology, NIMH; Laboratory of Clinical Science, NIMH; Laboratory of Psychology and Psychopathology, NIMH; Laboratory of Clinical Psychobiology, NIMH

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Biological Psychiatry Branch

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12	10	2

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☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Experimental and clinical findings have indicated that schizophrenia is a heterogeneous psychiatric disorder with biochemical, psychophysiological, psychological and genetic components. Pharmacological treatment is a particularly effective intervention, but not a cure for all patients. Because of its very nature, this disorder is difficult to study. Patients are not constantly psychotic and their clinical state may change, regardless of medication conditions. However, we are now able to study a schizophrenic patient population intensively and longitudinally, with and without pharmacological approaches during different clinical states. We are beginning to ask fundamental questions about the nature of schizophrenia, indications for pharmacological treatment, vulnerability to psychotic decompensation and the role that genetics may play in the transmission of the disorder.

In the United States alone, it is estimated that 2 million people suffer from schizophrenia. Our work integrates the multidisciplinary approaches in an effort to understand this complex disorder which may lead to a more effective treatment.

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Project Description

The Section on Neuropsychopharmacology (1) plans and conducts multidisciplinary research on the etiology and treatment of schizophrenia and (2) conducts research to define the biological dysfunction in schizophrenia.

Objectives

1. Multidisciplinary research on the etiology, treatment and prevention of schizophrenia
2. Evaluation of the dopamine, other amine and peptide hypotheses of schizophrenia
3. Evaluation of potential markers of vulnerability and predictors of antipsychotic drug response
4. Psychopharmacological and biochemical evaluation of trait- and state-dependent variables of schizophrenia
5. Evaluation of novel treatments such as hemodialysis.

Methodology

One to 14 days after admission, patients are taken off their neuroleptic medication and put on placebo. After this 2-week "washout period," patients go through a 3- to 4-week test period in which diagnostic evaluation, medical and neurological workup, cerebrospinal fluid (CSF) collections, drug infusions (amphetamine, naloxone, placebo), psychological and psychophysiological testing and neuroendocrine evaluations take place. These are followed by two or more drug trials which include pimozide, lithium, diazepam, GHB, lioresal, naltrexone or muscimol. During any of these drug trials, testing similar to the admission test week can take place. Each drug period is separated by a 2-week placebo "buffer" period. The last of this period is either followed by another test period or immediately followed by a 4-week clinical period where, if indicated, the patient is put on generally available antipsychotic medication and prepared for discharge. This extensive data-collection system allows us to evaluate the individual variables and drug responses against one another and to observe the patient in different clinical states. It will enable us to develop psychosocial, endocrine, biochemical, and pharmacological profiles on patient groups defined by each variable.

A major problem of schizophrenia research has always been certainty of diagnosis. In addition to using the International Pilot Study of Schizophrenia criteria as developed by the World Health Organization and the former Psychiatric Assessment Section of the Adult Psychiatry Branch, we are applying the Research Diagnostic Criteria as described by Dr. Spitzer and his collaborators to define diagnostically our patient population.

The 4-East Nursing Staff has been trained in the application of the Bunney-Hamburg psychosis, depression, and mania scale, and we have added a scale to measure extrapyramidal side-effects (EPS) and sedation.

Further, the scale of psychotic disorganization as designed by Drs. Docherty and van Kammen has been used by the 4-East Nursing Staff, and initial reliability and validity have been established. Understanding of the stages of phenom-

ena may strengthen our interpretation of biological data.

Hemodialysis of Schizophrenia

To evaluate in a double-blind manner the potentials of this experimental procedure, 4 patients have started on a 20-week hemodialysis (real and sham). A special cartridge has been developed to maintain the double blind. Patients will undergo drug-free testing, including CSF studies, endocrine and physiological testing. This will provide an opportunity for collaboration with investigators in other institutes who are interested in the effects of studying hemodialysis in a non-uremic population. (Drs. Balow, Schulz)

New Assays and Drugs

In collaboration with the Section on Biochemistry, BPB, and the Clinical Psychobiology Branch, new assays for measuring peptides in spinal fluid have been started. We presently have drugs available that are relatively specific for the different neurotransmitter systems that we are studying.

Major Findings: Pharmacological

A. Dopamine Stimulation

d-Amphetamine, an indirect dopamine receptor agonist, has been shown to worsen some acute psychotic symptoms. At the same time, numerous clinical reports of increased social contact and therapeutic effects have appeared in the psychiatric literature over the last 25 years. This, therefore, seems a potentially crucial area of investigation for clarifying the limits and nuances of the dopamine hypothesis. Patients receive I.V. amphetamine and I.V. placebo during the initial test period. This is repeated during pimozide withdrawal and/or lithium.

A standardized rating system has been developed for quantitating behavioral change before, during and after the infusion interview. Additionally, blood samples are taken regularly during the day to monitor amphetamine blood levels, serum prolactin, norepinephrine, and DBH levels. Clinically, schizophrenic patients appear to respond differently to amphetamine than depressed patients, although there is heterogeneity within each group (A paper has been published). Strikingly, the depressed patients do not become more psychotic, nor do patients with a schizophrenic diagnosis as a group. However, within this schizophrenic group the more severely psychotic patients may improve, while some patients in early remission may get worse. Serum norepinephrine is raised 15 minutes after the acute amphetamine administration. This rise over baseline is not affected by either pimozide or lithium. Serum DBH does not seem to be affected by amphetamine. This suggests that amphetamine affects newly synthesized, rather than stored, norepinephrine. However, there may be a competitive inhibition of DBH by amphetamine. Comparative animal data are not available. Lithium, which is supposed to prevent stimulation-induced release of norepinephrine, had no effect upon the amphetamine-induced rise in norepinephrine. A paper has been submitted for publication. Prolactin levels were increased 25 and 40 min. postamphetamine infusion. This was rather striking since the placebo infusions which took place at the same time of the day--between 8-9 a.m.-- showed a slight decrease from the rise of prolactin during sleep as a result of diurnal variation. This is in contrast to the

expected decrease in prolactin levels following increased dopamine activity. The increase is hypothesized to be due to stress or activation of serotonin mechanisms in the hypothalamus and pituitary.

B. Dopamine Receptor Blockade and Schizophrenia

(a) Pimozide in acutely psychotic patients - Following a washout period, patients are started in a double-blind, placebo-controlled trial of the effects of pimozide in acutely psychotic and remitted patients. This anti-psychotic agent is of especial interest because of its relatively specific postsynaptic dopamine receptor blocking effect.

During this project endocrine studies, amphetamine infusion, sleep and CSF studies are conducted. Selected patients are put on sleep study to evaluate the effects on sleep. In collaboration with Dr. Gillin we have shown that pimozide prevented amphetamine's having any effect on sleep. Rapid eye movement sleep decrease is completely prevented by pretreatment with pimozide. This is in contrast to the behavioral effects of amphetamine, which are reversed in the people who improve while drug-free after amphetamine and who then, after pimozide, become more psychotic. We also gave amphetamine infusions seven days after pimozide discontinuation to evaluate whether receptor hypersensitivity could be connected with a change in behavioral response. Data from this project are presently under analysis. However, we did not observe a strong response to amphetamine, which questions the dopamine hypersensitivity hypothesis of psychosis. A paper evaluating the effects of pimozide on sleep in psychiatric patients has been accepted for publication, as well as a paper on the pimozide attenuation of the amphetamine-induced REM suppression.

Other studies include endocrine studies, cyclic AMP in CSF, effects of pimozide on DBH in spinal fluid, metabolites of dopamine, norepinephrine and serotonin in the spinal fluid and 24-hour urine collections which take place in addition to the standard battery of behavioral ratings. In collaboration with Dr. Heykants of Beerse, Belgium, we are measuring pimozide levels in our patients with a radioimmunoassay. These levels will be examined in relationship to the above-mentioned hormones and urinary free cortisol. In a first group of 13 patients the increase in prolactin observed after pimozide and the decrease in psychosis were correlated. However, in a second group of five male patients, this was not observed.

C. Use of Antidepressants in Schizophrenia

(a) An extensive review of the literature was conducted to ascertain the experience which has been accumulated in using tricyclic antidepressants and monoamine oxidase inhibitors in the varied types of schizophrenia. Although the number of well-controlled studies in this area is limited, it is well-documented that antidepressant medications are widely prescribed for these patients. A paper has been accepted for publication.

We observed that eight of a group of 24 psychotic patients, whom we were not able to differentiate among clinically on admission, remitted when their anti-psychotic medication was withdrawn over a 30-day period. Although these patients all had been diagnosed schizoaffective schizophrenia according to RDC classifi-

cation (1975), this diagnosis did not differentiate the drug-free remitters from the schizoaffective nonremitters. The drug-free remitters appeared to have a later age of onset, a shorter time of hospitalization and better premorbid functioning. The drug-free remitting male patients had low platelet MAO activity below the mean for normals, while the nonremitting patients had platelet MAO activity ranging from high to low. The drug-free remitters also responded to lower than the suggested dose ranges for the nondrug-free remitters. This continued to be present at one-year follow-up (150 vs. 484 chlorpromazine units/day). This finding may lead to a differential approach of medicating acutely psychotic patients. It suggests that if acutely schizophrenic patients have not responded with improvement to neuroleptic drugs at 4-6 weeks, the dose may need to be decreased rather than raised.

D. Lithium in Schizophrenia

Since the behavior activation induced by amphetamine also was attenuated by it, lithium may have some antidopamine effects. We studied lithium carbonate in psychotic and remitted schizophrenic patients: Nine of thirteen psychotic patients responded with an improvement either in psychosis or depression. Diagnostic categories did not separate the responders from the nonresponders. Six of 11 remitted patients who showed postpsychotic depression improved on lithium. We are now studying biochemical and other biological variables to determine which of these may predict a response to lithium. Four papers have been submitted for publication. Dr. van Kammen was invited to the 1978 meeting "Lithium: Controversies and Unresolved Issues", to review lithium in schizophrenia.

We examined serum calcium (Ca) and magnesium (Mg) levels in patients before and during neuroleptic treatment (23 patients). Low normal Ca and Mg was an indication of emergence of EPS. Differences in Ca and Mg in drug-free schizophrenic patients were observed between diagnosis and clinical states. One paper has been accepted and another has been submitted for publication.

E. Gamma-Aminobutyric Acid and the Dopamine Hypothesis of Schizophrenia

(a) A hypothesis has been developed about an ineffective feedback system regulating dopamine in psychosis. Gamma-aminobutyric acid (GABA) seems to be implicated in this role. A review paper has been published.

(b) Diazepam, one of the first compounds found to increase GABA function in the brain, was tried on our patients. We administered diazepam in doses higher than 40 mg per day to five patients. Only two severely psychotic patients received 300 mg of diazepam with equivocal results. One patient, who was still relatively nonpsychotic following pimozide withdrawal, became acutely psychotic when he was withdrawn from 70 mg of diazepam. The more psychotic the patient, the less he was bothered by side-effects like ataxia and sedation.

(c) GABA levels in CSF of schizophrenic patients are presently under study in collaboration with Drs. Post, Bunney and Pert, as well as with Dr. Hare.

(d) Studies with the GABA agonist muscimol have begun.

F. Endocrinological Project

(a) Evaluation of baseline hormonal levels in schizophrenic patients is being conducted, focusing on hormones that are under total or partial control of dopamine. Prolactin, Growth hormone, FSH, and LH are measured in serum. Measurements have thus far been made with placebo, chronic pimozide GHB, and propranolol. Increase in prolactin levels follows dopamine blockade (by pimozide) as expected. A report was published in the Psychopharmacology Bulletin.

(b) We also examined and studied the growth hormone and prolactin response to apomorphine in drug-free psychotic patients. Patients participated three days after neuroleptic withdrawal, after three weeks placebo, and one day after GHB withdrawal. These data are presently under analysis.

(c) Chronic treatment with pimozide increased prolactin, decreased LH.

G. Cerebrospinal Fluid Studies (CSF) in Schizophrenia

Baseline and probenecid accumulated metabolites of dopamine, norepinephrine, serotonin, tyramine and phenethylamine in CSF are being measured with mass spectrograph.

In 12 patients HVA was evaluated twice during their stay in drug-free conditions--initially, when they were psychotic and four months later when they had improved. During their second drug-free lumbar puncture, HVA appeared to be decreased as was the psychosis.

Early evaluation of pimozide effect upon HVA showed that eight responders had a subsequent rise of HVA but three nonresponders had a decrease. Further patient data have not supported this early observation.

Measurement of viral antibody titers in CSF has been temporarily discontinued.

CSF studies for c-AMP and c-GMP - Mean values were not different from depressed comparison groups of patients. Probenecid increased the baseline values. The effects of pimozide are presently under study.

A study of pterin cofactor activity in our patients and in normal comparison groups has started (R. Levine, W. Lovenberg and J. Ballenger).

CSF electrolytes. Dr. Jimerson submitted a paper showing that remitted schizophrenic patients had higher CSF Ca than when psychotic.

Evaluation of peptides in CSF has been started (somatostatin, vasopressin).

H. Dopamine Receptor Blockade Studies

Dr. Rosenblatt started ^{3}H-DA receptor and ^{3}H-spiroperidol receptor studies to measure dopamine blockade in serum of patients on neuroleptics (e.g., pimozide). The collection and assay of the serum is presently under way.

H. Monoamine Oxidase (MAO)

Monoamine oxidase (MAO) in plasma or platelets has been implicated in vulnerability to chronic unremitted schizophrenia and affective disorders. We are presently evaluating clinical and biochemical variables to activity levels. Preliminary data in our male drug-free remitted schizophrenic patients indicate lower than the mean compared to a normal control population. A brief communication has been published.

I. Norepinephrine

(a) DBH and norepinephrine are measured in the serum of our drug-free patients and while they are on lithium and on pimozide. Pimozide nor lithium seem to affect circulating serum levels of both substances.

(b) DBH in CSF of schizophrenic patients show similar levels to that of normals. Dr. Sternberg presented our data at the APA. Patients with poor premorbid functioning tended to have higher DBA levels. CSF NE levels are presently being measured by Dr. Lake.

(c) Dr. Tallman measured β-norepinephrine receptors on leucocytes from psychotic and nonpsychotic patients. There appears to be no difference between schizophrenic and normal controls while the receptor count stays stable over time. A paper is in preparation.

(d) Dr. Marian Kafka measured α-receptor activity in our drug-free patients. Although the α-receptor activity is not different in schizophrenic patients compared to normal controls, in the male patients c-AMP production was 50% less than in the controls. A paper was presented at the APA meeting in Atlanta, May 1978.

(e) A clinical evaluation of d- and dl-propranolol is under way with concomitant measurement of plasma levels, β-receptor binding, endocrines and CSF metabolites.

J. Endorphins

(a) A hypothesis that endorphins (e.g., enkephalins) may be involved in schizophrenia. A study was undertaken to evaluate a potential therapeutic effect of an opiate antagonist, naloxone, in psychosis. The study was done in collaboration with the Laboratory of Clinical Psychopharmacology, Special Mental Health Research at St. Elizabeths Hospital with equivocal results.

(b) In collaboration with Drs. C. Pert, Post, and Bunney we are measuring enkephalin CSF levels in drug-free psychotic and remitted schizophrenic patients and after pimozide.

(c) A study with a longer acting opiate antagonist, naltrexone, has started. Initial results have been reported by Dr. Davis. Equivocal effects were observed in two patients.

Major Findings: Psychophysiological

A. Laterality Hypothesis

The Section has continued to test the "laterality" hypothesis. This represents a novel approach to the biology of psychosis. The hypothesis is based on the demonstrated cognitive specialization in man of the cerebral hemispheres. We speculate that in psychosis there is an inappropriate intrusion of right-sided processes. To develop some relevant measures, 16 normals have been tested for differential lateralized cerebral response under a variety of stimulus conditions. A paper has been submitted for publication. These results will be compared with other clinical, biochemical, and pharmacological measures.

B. EEG Recording of Sleep

Sleep studies in relationship to amphetamine and pimozide have been undertaken. Pimozide appears to prevent the amphetamine-induced REM suppression. (See Pharmacological findings)

C. Psychophysiological Testing

Dr. Zahn of the Laboratory of Psychology and Psychopathology is testing our patients in drug-free and during different drug conditions.

Major Findings: Behavioral and Psychological

A. Drug-free Improvement

Following neuroleptic withdrawal, we observed improvement in 7 patients out of 24 psychotic schizophrenic patients. Platelet MAO tended to be lower in these relatively "good progress" patients.

B. Hemodialysis

So far one patient has finished 18 dialyses, including 10 active ones, without much behavioral change.

C. Information-processing Project

(a) A simply administered, objective, quantified measure of cognitive disorder has been developed. Preliminary results have disclosed a highly significant difference between patients and normals. We will use this technique to investigate the effects of psychoactive drugs and state variables on schizophrenic thought disorder. We are also investigating the presence and degree of thought disorder in the families of schizophrenic patients which appeared to be shared by at least one parent in all our studied patients. A paper has been accepted for publication.

(b) Schizophrenic thought disorder is also being investigated with a battery of psychological tests mainly derived from standard I.Q. tests; some scores appear to be impaired in schizophrenia. This emphasizes the need for a

special psychological evaluation report.

Major Findings: Genetic

A. HL-A Typing Acute Schizophrenia

HL-A antigens are genetically determined human cell membrane antigens found on a variety of cell types. The genes controlling their expression are found on chromosome 6 and are thought to be linked closely to genes controlling immune responses to a number of antigens. In recent years associations of certain HL-A types and certain diseases have been found. These diseases are frequently those with associated or causal disorders of immunity, possibly the result of aberrant immune-response genes--thus their association with HL-A.

Schizophrenia may have causal or secondary immune abnormalities; therefore, a study of HL-A antigen frequencies in this disease seemed worthwhile. If found, this would constitute more evidence for the role of the immune system in schizophrenia. To date we have typed 75 patients with schizophrenia.

B. Catechol-O-methyltransferase in red cells has been measured in collaboration with Dr. Gershon. Data are being analyzed.

Significance to Mental Health Research

These studies contribute to the understanding of a complex psychiatric disorder from which two million Americans are suffering. Our work integrates the multidisciplinary approaches of this disorder. Receptor studies in man have only recently started. Alpha- and beta-adrenergic receptors appear to be normal.

Pimozide attenuated the amphetamine-induced sleep changes indicating a role for dopamine in the observed sleep disorder. This may have implications for narcolepsy, a disorder in which amphetamine increases wakefulness. Amphetamine raised norepinephrine acutely as well as blood pressure and pulse, effects that were not affected by lithium or pimozide.

Hemodialysis: The claims of miraculous cures are under investigation. To date (June 1978) we are still the only group who studies this procedure in double-blind fashion. However, in many places in this country, hemodialysis is being started as a treatment. This project will allow for extensive study of biochemical correlates if behavioral changes occur.

Lithium may be of help in the management of schizophrenic patients, although it is less effective than neuroleptics.

Serum calcium appears to be lower in schizophrenics after chronic pimozide.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00081-04 BP

PERIOD COVERED
October 1, 1977 - September 30, 1978

TITLE OF PROJECT (80 characters or less)
Heritable Characteristics of Cation Transport in Primary Affective Disorders

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	A. Frazer, Ph.D., Univ. of Penn. School of Medicine	

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Section on Psychogenetics

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.2	0.1	0.1

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The principal area of focus for this project is the identificaton of heritable characteristics of ion transport which may distinguish individuals with a primary affective disorder from normal controls.

The lithium plasma/erythrocyte ratio has been examined along with other parameters as possible predictors of lithium response during depression. High platelet monoamine oxidase and "augmentation" of visual average evoked response were found to be the best predictors. Lithium plasma erythrocyte ratio was not found to be a good predictor.

A study of sodium potassium stimulated adenosine triphosphatase (Na^+-K^+-ATPase), a cation transport enzyme, is beginning. An assay is being developed to determine the level of this membrane enzyme in bipolar and unipolar depressed patients and in normal controls. A twin study will serve to determine heritability of this enzyme.

PHS-6040
(Rev. 10-76)

Project Description

It has been reported that lithium antidepressant response is significantly correlated with the rate of transport of the lithium ion across the red cell membrane. We have examined this parameter in conjunction with a number of other biological and clinical variables, in a study of 43 patients treated with lithium on NIH inpatient wards. Lithium plasma/erythrocyte ratio (a measure of lithium transport) was not found to be highly correlated with antidepressant response. "Augmentation" of average evoked response (AER) and platelet monoamine oxidase (MAO) were found to be highly correlated with lithium response ($p<.01$). Multiple regression analysis showed these parameters, along with high dopamine beta hydroxylase (DβH) to account for 57% of the variance in response to lithium. All factors taken together predicted over 90% of the variance.

A study of Na^+-K^+-ATPase as an indicator of cation transport is beginning. A colorimetric assay that can detect in the range of 10^{-9} moles of inorganic phosphate is being developed. It is planned to test the heritability of this enzyme in a group of normal twins, and then to compare normal values to those in patients with unipolar or bipolar affective disorder.

Patients will be followed with enzyme levels over time and in different mood states. It has been reported previously that this enzyme is decreased during depression.

Significance to Mental Health Research

Lithium treatment is highly effective in affective disorder, but carries certain risks and complications. A biological criterion for probable lithium response would be of significant value to clinicians. Identifying populations of responders is also helpful in delineating biologic differences in subpopulations of affectively ill patients.

Cation transport mechanisms depend on cell membrane characteristics that are likely to be largely genetically determined. Previous studies have demonstrated differences in cation transport mechanisms in affectively ill patients. One transport mechanism, lithium plasma/erythrocyte exchange has not been found in our studies to be correlated with lithium response or to distinguish bipolar from unipolar patients. Another mechanism, the sodium potassium stimulated ATPase, is now being investigated.

Publications

Nurnberger, J.I., Gershon, E.S., Murphy, D., Buchsbaum, M., Goodwin, F.K., Post, R., Lake, R., Guroff, J., McGinniss, M. Biological and clinical predictors of lithium response in depression. Proceedings of International Lithium Conference 1978. In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-MH 00082-04 BP

PERIOD COVERED
October 1, 1977 through September 30, 1978

TITLE OF PROJECT (80 characters or less)

Life Events, Social Support and Alienation in Affective Disorders

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	E. D. Dibble, D.S.W.	Psychiatric Social Worker	BPB NIMH
OTHER:	E. S. Gershon, M.D.	Chief, Section on Psychogenetics	BPB NIMH
	M. M. Weissman, Ph. D.	Depression Research Unit	Yale University
	S. D. Targum, M.D.	Clinical Associate	BPB NIMH
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COOPERATING UNITS (if any)
Depression Research Unit, Yale University

LAB/BRANCH
Biological Psychiatry Branch

SECTION
Section on Psychogenetics

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.2	0.1	0.1

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this investigation is to measure the relationship between the number and kind of life events, the amount of subjectively felt social support and social alienation to mood disorders and to changes in specified biochemical levels in affectively disturbed patients vs. controls. The Life Events Scale, adapted from earlier research is being used in conjunction with the family study interview and blood studies of patients and controls and their family members. In addition, a new scale, the Family Attitudes Questionnaire, has been developed to measure patients' and relatives' perceptions of the morbid risk for depression or mania of the burden these disorders create for the family, and the affect this may have on plans for marriage and child-bearing.

PHS-6040
(Rev. 10-76)

Objective:

1. To collect standardized data on life events, social support and social alienation.

2. To compare these data with biological data for the same subjects.

3. To compare the data from a large group (approximately 100) of patients with that from matched controls.

4. To compare a) the number and kind of life events between patient and non-patient populations; b) the concentration of life events; c) the family culture associated with life events for either resident or non-resident family members in contrast to controls.

5. To correlate the number and stress of life events with the biochemical levels of DBH, COMT, MAO, etc. obtained on this same sample population.

6. To compare differences in the amount of social support perceived by patients and non-patients.

7. To compare the degree of social alienation of patients and non-patients.

8. To compare patients and relatives' attitudes toward a) the most troublesome characteristics of the acute stages of mania and depression and b) the outgoing social problems caused by the disorders and their perception of (a) the causes for the disorders; (b) the degree of risk in the general population vs relatives of patients (with mania or depression), (c) how much burden these disorders cause in comparison to various medical conditions.

9. To assess patients and relatives' attitudes toward marriage and childbearing as an adjunct to therapeutic intervention or for genetic counseling.

Method:

Data on life events, social support and social alienation is being collected by use of the Life Events Scale (based on the earlier work of Holmes and Rahe, Cochrane, Srole, among others). The Family Attitudes Questionnaire, a newly devised scale, is being used to collect data on the perceptions and attitudes toward mania and depression.

Both scales have been incorporated in the interview during which diagnostic, family history, and blood sample data is obtained from both patients and their first degree relatives.

Frequency calculations, correlations, factor analysis, cluster analysis (to test relationships), and analysis of variance will be used to analyze the data when it has been completed.

Progress to date:

Life event scales have been given to 179 patients who are part of the larger family study of affective disorders. The scales have been, or are being, completed by their first degree relatives. During the reporting year the Life Events Scale has also been used by the controls in the New Haven community.

The new scale, the Family Attitudes Questionnaire, has been used on a pilot sample of 19 manic-depressive patients and their spouses. Results of this pilot study have been used to refine the instrument which will now be used to increase the sample of married patients, divorced patients and their spouses, when possible, never-married adult patients, well relatives of patients.

The Family Attitudes Questionnaire will also be used by patient-relative populations in other than NIMH to assess whether perceptions and attitudes vary with the setting.

Significance to Mental Health Research:

Although research on life events has already been researched for patients with a variety of diagnoses, the family culture of life events, the impact of social support or its lack, and alienation from society have not previously been related to the affective disorders. Furthermore investigations have not previously correlated findings on life events and stress with the various biological findings in this study.

The Family Attitudes Questionnaire provides a new instrument for use in supplementing other methods of assessing patients and relatives' attitudes toward mania and depression and the influence these may have on what they do. It offers a tool for both aiding in therapeutic intervention when episodes occur and in prevention in the sense of minimizing the impact of illness by educating individuals to recognize symptomatology in the early stages and warding off greater severity.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00083-04 BP

PERIOD COVERED
October 1, 1977 through September 30, 1978

TITLE OF PROJECT (80 characters or less)

Hypothesis of X-linkage in Bipolar and Unipolar Affective Disorder

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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OTHER:	W. E. Bunney, Jr., M.D.	Chief BPB NIMH
	S. Matthysse, Ph. D.	Associate Professor Harvard Univ.
	S. D. Targum, M.D.	Clinical Associate BPB NIMH
	J. F. Leckman, M.D.	Resident in Psychiatry Yale Univ.
	M. McGinniss	Research Biologist CC BB
	H. Hippius, Ph. D.	Munich, West Germany
	P. Kielholz, Ph. D.	Psychiatrische Clinic Univ. Basel, Basel, Switzerland
	J. Mendlewicz, Ph. D.	Institut de Psychiatrie Brussels, Belgium
	O. Rafaelsen, Ph. D.	Psychochemistry Institute Copenhagen, Denmark

COOPERATING UNITS (if any)
Yale University; Harvard University; Blood Bank, Clinical Center, NIH; World Health Organization centers for collaborative study

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1.0	0.5	0.5

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☐ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Mathematical genetic models of X-chromosome transmission and studies of genetic linkage to X-chromosome markers are being performed in primary affective disorders. Using newly developed mathematical models, linkage of bipolar manic depressive illness to the regions immediately surrounding the color blindness loci and the Xg blood group antigen locus may be definitively rejected.

PHS-6040
(Rev. 10-76)

Other Investigators Continued:

H. Van Praag, Ph. D.	Univ. of Groningen Groningen, Netherlands
M. Vartanian, Ph. D.	Academy of Med. Sciences, Moscow, U.S.S.R.
J. Avni, M.D.	Hadassah Univ. Hospital, Jerusalem, Israel
H. Beckmann, M.D.	Munich, West Germany
M. Gastpar, M.D.	Psychiatrische Clinic Univ. Basel Basel, Switzerland
R. Rosenberg, M.D.	Psychochemistry Institute Copenhagen, Denmark

Project Description:

Objectives:

1. To develop new data pertinent to the hypothesis that some affective disorders are transmitted as a single gene on the X-chromosome. This hypothesis was given great impetus by the studies of Mendelwicz, Fieve and Fleiss, in which linkage to both the Xga blood group and protan and deutan color blindness was reported.

2. To develop and test appropriate mathematical models of X-linked inheritance in psychiatric illness, and apply them to existing and newly collected data.

Methods Employed:

1. Patients with Bipolar or Unipolar affective disorder and their first degree relatives (parents, sibs, children) are studied for psychiatric status, color blindness and for Xg blood type. If it is determined that a family is genetically informative for Xg or color blindness, more distant relatives are studied until a large pedigree with multiple informative sibships is obtained. Analysis is performed using the method of lod scores and using multigeneration models.

2. Mathematical models have been developed for liability to a psychiatric disorder, with multiple thresholds according to sex and form of disorder found (bipolar, unipolar, or related disorders.) These are then applied for goodness-of-fit to existing and currently collected data on prevalences in relatives.

3. New methods of genetic analyses are being developed because psychiatric illness is different from classical genetic markers, in the multiple manifestations of disorder, variable age of onset, penetrance, heterogeneity of disease entities and methods of case ascertainment.

Major Findings:

1. The major methodological advance is the acquisition of the LIPED soft ware for analysis of multigenerational pedigrees, and our modification of it to the peculiar conditions of psychiatric illness, which are variable penetrance as a function of age and other factors. This system is superior to the method we developed for analysis of pedigrees in which more than two generations are found, but is equivalent to the methods we have developed for two generation pedigrees.

Using this program to obtain precise estimates of the probability of linkage, bipolar illness can be definitively excluded from a region of the X-chromosome immediately surrounding the color blindness locus, and from another region containing the Xg antigen.

More data would be needed to exclude linkage to areas more distant from the two characteristics (Xg antigen and color blindness) studied. This will be provided in the case of Xg antigen by further data that we are developing. In the case of color blindness, data from the World Health Organization (WHO) collaborative study group may prove useful. We are currently going over pedigrees supplied by collaborators of that group for methodological standardization.

Proposed Course of Project:

Data collection at NIMH is substantially complete, and should be ended during the 1978 fiscal year. Application of further mathematical models to the data will proceed during the following year.

Significance of These Findings:

This project is the first study to simultaneously apply advance techniques of mathematical modeling and of family study to the question of linkage of a chromosomal marker to a psychiatric illness. This study incorporates (1) family study methodology using demonstrably reliable criteria for diagnosis and well standardized interviews, (2) mathematically appropriate methods of analysis for linkage to an illness with variable penetrance. We do not replicate previous reports of linkage of bipolar illness to these X-chromosome markers, possibly because of the more careful methodology used in the present studies.

The attempt to separate out an X-linked form of affective disorder is a most important and powerful investigative strategy. If the hypothesis were confirmed, it would constitute proof of a genetic factor in the mood disorders. It would also allow more complete definition of the biological and psychological effects of an "affective disorders genotype" than has been possible until now. For these reasons, this hypothesis must be subjected to the most rigorous possible tests and methods of analysis.

Publications:

1. Gershon, E.S., Matthysse, S.: X-linkage: Ascertainment through doubly ill probands. J. Psychiat. Res. 13:161-168, 1977.

2. Gershon, E.S.: The search for genetic markers in affective disorders. In DiMascio, A. (Ed.): Psychopharmacology: A Generation of Progress. New York, Raven Press, 1977, pp. 1197-1212.

3. Leckman, J.F., and Gershon, E.S.: Autosomal models of sex effect in bipolar-related major affective illness. J. Psychiat. Res. 13:237-246, 1977.

4. Gershon, E.S., Targum, S.D., Kessler, L.R., Mazure, C.M. and Bunney, W.E., Jr.: Genetic studies and biologic strategies in the affective disorders. Progress in Medical Genetics, New Series, 2:101-164, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00084-04 BP

PERIOD COVERED

October 1, 1977 through September 30, 1978

TITLE OF PROJECT (80 characters or less)

Genetic-Biologic Studies of Psychiatric Disorders

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	L. Cytryn, M.D.	Medical Officer, Research	BPB NIMH
	R. O. Rieder, M.D.	Research Psychiatrist	LPP NIMH
	M. M. Weissman, Ph. D.	Associate Professor	Yale University
	K. Kidd, Ph. D.	Assistant Professor	Yale University
	R. J. Wyatt, M.D.	Chief, Clin. Psychopharmacology	SMRC NIMH
	S. Zalcman, M.D.	Clinical Associate	SMRC NIMH
	D. McKnew, M.D.	Medical Officer, Research	BPB NIMH
	M. Buchsbaum, M.D.	Chief, Unit on Perceptual and Cognitive Studies	BPB NIMH

COOPERATING UNITS (if any)

Immunology Branch, DCBD, National Cancer Institute; CPB, NIMH;
Division of Medical Genetics, UCLA School of Medicine; CNB, NIMH
Yale University

LAB/BRANCH

Biological Psychiatry Branch

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Section on Psychogenetics

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NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.7	2.1	1.6

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☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A family study of primary affective disorders is being performed. Biologic characteristics of the patients are evaluated for usefulness as genetic markers of vulnerability to affective disorders. Platelet monoamine oxidase is not a marker of vulnerability within pedigrees, although it is associated with increased risk of illness in relatives. Genetic linkage of affective disorders to known chromosomal markers is also studied. Histocompatibility Leukocyte Antigens (HLA) are not associated with Bipolar affective illness and are not markers of vulnerability within pedigrees. Study of specific genes in pedigrees revealed a previously undocumented gene, a null allele for esterase D. Dopamine beta hydroxylase (DBH) is not transmitted as an autosomal recessive. Heterozygosity for phenylketonuria (PKU) is not increased among bipolar patients. Mathematical models of genetic transmission have been developed for application to these data. Clinical applications of genetic findings to prediction of pharmacologic response, case finding in the population, and genetic counseling have been developed.

Other Investigators Continued:

J. I. Nurnberger, M.D.	Sr. Staff Fellow	BPB NIMH
J. F. Leckman, M.D.	Resident in Psychiatry	Yale University
E. D. Dibble, D.S.W.	Social Worker	BPB NIMH
N. Rogentine, M.D.	Senior Investigator	NCI
R. S. Sparkes, M.D.	Chief, Div. Medical Genetics	UCLA
M. A. Spence, Ph. D.	Associate Professor	UCLA
D. L. Murphy, M.D.	Chief	CNB NIMH
W. Sceery, M.S.W., J.D.	HSO Director	BPB NIMH
S. D. Targum, M.D.	Clinical Associate	BPB NIMH
C. M. Mazure, M.S.	Psychologist	BPB NIMH
C. W. Abell, Ph. D.	Prof. of Molecular Biology	Univ. of Texas

Major Findings:

1. Identification of Etiologic Genetic Markers in Affective Disorders

A. Platelet Monoamine Oxidase (MAO). The method of Rieder and Gershon tests whether a biologic characteristic is transmitted in pedigrees along with illness. Reduced platelet monoamine oxidase activity in bipolar patients has previously been reported. This finding was replicated for both male and female Bipolar I patients, but not Bipolar II patients. Selecting out those Bipolar I patients who have low platelet monoamine oxidase, we examined their relatives and found that MAO activity in their relatives did not distinguish the ill relatives from the well relatives. Therefore, this characteristic is not a marker of vulnerability within pedigrees. Similar studies are underway for catechol-O-methyltransferase, and dopamine beta hydroxylase.

B. Predictors of Increased Morbid Risk in Relatives. A second approach to markers of vulnerability is to identify biologic characteristics in patients associated with increased family history of illness. Patients with reduced platelet MAO activity do have slightly more illness in their relatives, as do patients with increased DBH activity. Anti-depressant response to lithium is also associated with increased illness in relatives. Using multiple regression techniques, about 40% of the variance in family history of illness is predictable by biologic markers and clinical variables in the patients. We are applying this finding to the question of genetic transmission of these biologic characteristics in the vulnerability to illness.

C. Identification of Increased Prevalence of Known Chromosomal Markers in Patients with Affective Disorders as a Third Strategy to Identifying Genetic Markers of Vulnerability. Approximately thirty known linkage markers are studied in pedigrees of bipolar patients. Particular HLA phenotypes have been associated by different investigators with patients who have bipolar manic-depressive illness, although no two investigators have replicated each others findings. We have demonstrated that artifactual associations may result from slightly different ethnic backgrounds of patients and control groups. The HLA antigen Bw16, which had the strongest reported association with Bipolar illness, is a population marker for Ashkenazi Jews.

We have compared 21 Ashkenazi Jewish Bipolar patients from our clinic with 552 Ashkenazi Jewish controls in Israel, and found that although 38% of the Jewish patients had the Bw16 genotype, this was not significantly different from the frequency in Jewish controls. Similarly, our non-Jewish patients had no more Bw16 than did a control sample of white persons of European (but not predominantly Jewish) extraction in North Carolina.

Further studies were performed to determine if linkage of transmission of HLA antigens to transmission of Bipolar illness in some small number of pedigrees might be present. Nine two-generational or multigenerational families were studied. Linkage of HLA transmission to bipolar illness transmission could be clearly ruled out in this series. This is the first pedigree series studying HLA and bipolar illness presented to the scientific community.

Other studies of alleles at specific loci have been performed. We have demonstrated that heterogosity for phenylketonuria (PKU) is not present in elevated frequency in patients with affective illness. A previously unproven gene (a null allele for esterase D) has been demonstrated in one of our pedigrees, but it is unrelated to affective illness. Dopamine beta hydroxylase (DBH) activity has been reported to be transmitted as a simple autosomal recessive gene. Our pedigree data do not suggest that this is true.

2. Methodological Advances

The problem of genetic analyses of multigenerational pedigrees for an illness with variable penetrance has only recently become tractable. We have recently acquired the LIPED computer program for these analyses from Prof. J. Ott at the University of Washington. After having modified it to deal with the particular penetrance problems of manic depressive illness, it appears far superior to previously existing computer programs for analysis of chromosomal linkage markers. It is now being used in all linkage analyses in this section.

3. Family and Pedigree Studies of Affective Disorders

For testing hypothesis of mode of genetic transmission and linkage to chromosomal markers we are conducting a large family study of affective disorders with direct examination and collection of biologic data from large numbers of relatives. To date we have examined 101 bipolar I patients, 28 bipolar II patients, 46 unipolar patients, 10 schizo-affective patients, 1119 relatives and 372 controls.

A collaborative family study of affective disorders is on-going between the Section on Psychogenetics and the Depression Research Unit headed by Dr. Myrna M. Weissman at Yale University, where Drs. Weissman and Kenneth K. Kidd are the collaborators. We have carefully standardized interview and diagnostic methods with these collaborators. Most recently, we have had six patients and relatives interviewed at separate times by teams from our center

and from the Yale center. All six persons thus interviewed had good diagnostic reliability between centers. They will collect data on 50 hospitalized unipolar patients, 100 non-hospitalized unipolar patients and 100 controls, and four first-degree relatives of each of the 250 probands plus additional relatives who are of interest.

We anticipate that at the time that this study is completed it will represent the largest controlled family study of mood disorders performed to date, and will enable much more precise testing of genetic and biologic-genetic hypotheses in the affective disorders.

Personality factors in the transmission of affective disorders are being studied using the Marke-Nyman scale and the Maudsley Personality Inventory.

4. Childhood in Families of Patients with Mood Disorders

The prevalence of psychiatric illness during childhood in the offspring of our patients is being studied in collaboration with the Unit on Childhood Mental Health Studies. Children are examined directly by that unit as described in another report (#Z01 MH 00098-04 BP). Preliminary studies indicate that depression is significantly more prevalent in the children whose parents or grandparents have major affective disorders than in control children.

Since the number studied in this way is small, we are also doing a family history study surveying all probands in the NIMH cohort and in the collaborating cohort at Yale where there are children currently less than 18 years of age.

5. Clinical Applications

The application of genetic findings in methods to identification of cases in the population, prediction of pharmacologic response, and in genetic counseling appear promising. We have demonstrated that identification of new and untreated cases of major affective illness in the population is achieved at least five times more efficiently using family study methods on all first degree relatives, including those at great distance, than by population survey methods. Of particular interest is our finding that we are able to reach relatives who are otherwise outside the medical and psychiatric care systems.

Prediction of anti-depressant response to lithium is associated with family history of affective illness and of other major psychiatric disorders, as discussed in a separate annual report. Using genetic and biologic variables, we are able to account for 92% of the variance in treatment response among patients.

Genetic counseling is requested of us not infrequently, and the belief is wide spread that genetic factors are a cause of affective illness. We have

identified in patients and spouses perceptions of risks to relatives, and burden of illness using a Family Attitude Questionnaire (FAQ) described in a separate annual report (#Z01 MH 00082-04 BP). This information, combined with development of principles of genetic counseling for use in psychiatry, has clear clinical implications.

Publications:

1. McKnew, Jr., D.H., Cytryn, L., Efron, A.M., Gershon, E.S. and Bunney, Jr., W.E. Offspring of Patients with Affective Disorders. Br. J. Psychiatry. In press.

2. Mazure, C. and Gershon, E.S. Blindness and reliability in lifetime psychiatric diagnosis. Archives of General Psychiatry. In press.

3. Gershon, E.S.,Targum, S.D., Leckman, J.F., Guroff, J.J. and Murphy, D.L. Platelet Monoamine Oxidase (MAO) Activity and Genetic Vulnerability to Bipolar (BP) Affective Illness. Psychopharmacology Bulletin, In Press.

4. Gershon, E.S. Genetics and Man. In Insel, P.M. and Roth, W.T. (Eds.). Health in a Changing Society Mayfield Publishing Co. Palo Alto, Second Edition. To be published.

5. Gershon, E.S. and Liebowitz, J.M. Affective Disorders in Jews. In Goodman, R.M. and Motulsky, A.G. (Eds.) Genetic Diseases in Ashkenazi Jews, Raven Press, N.Y. To be published.

6. Rieder, R.O., Gershon, E.S. Genetic Strategies in Biologic Psychiatry. Arch. Gen. Psychiatry. Accepted for Publication.

7. Buchsbaum, M.S., Gershon, E.S. Genetic Factors in EEG, Sleep and Evoked Potentials. In Human Consciousness and its Transformations: A Psychobiological Perspective, R.J. Davidson, J. Davidson, C.E. Schwartz (Eds.) Plenum Press, N.Y. Accepted for publication.

8. Gershon, E.S., Matthysse, S.: X-Linkage: Ascertainment through doubly ill probands. J. Psychiatr. Res. Vol. 13, pp. 161-168, 1977.

9. Gershon, E.S., Targum, S.D., Kessler, L.R., Mazure, C.M., Bunney, W.E. Bunney, W.E. Jr. Genetic studies and biologic strategies in the affective disorders. Progress in Medical Genetics, New Series, Vol. II, 101-164.

10. Gershon, E.S. The search for genetic markers in affective disorders. In Psychopharmacology: A Generation of Progress. DiMascio, A. (ed.) Raven Press, N.Y. pp. 1197-1212, 1977.

11. Leckman, J.F., Gershon, E.S., Autosomal models of sex effect in bipolar-related major affective illness. Journal of Psychiatric Research. Accepted for publication.

12. Gershon, E.S., Kessler, L.R. and Bunney, W.E., Jr. Monoamine Enzymes and Inhibitors in Affective Illness. In Structure and Function of Monoamine Enzymes, Osdin, B., Weiner, N. and Y oudim, M.B.R. (Eds.) Dekker, N.Y., 1977, pp. 905-939.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00085-02 BP

PERIOD COVERED

October 1, 1977 - September 30, 1978

TITLE OF PROJECT (80 characters or less)

Pharmacogenetics of Psychoactive Drugs

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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Other:	William Bunney Jr., M.D.	Chief	BPB	NIMH
	Monte Buchsbaum, M.D.	Chief, Unit on Perceptual and Cognitive Studies	BPB	NIMH
	Michael Ebert, M.D.	Chief, Section of Experimental Therapeutics	LCS	NIMH
	Raymond Lake, M.D.	Research Psychiatrist	LCS	NIMH
	John Tallman, Ph. D.	Section on Biochemistry and Pharmacology	BPB	NIMH
	John Nurnberger, M.D.	Clinical Associate	BPB	NIMH

COOPERATING UNITS (if any)

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LAB/BRANCH

Biological Psychiatry Branch

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The inheritance of variability in the behavioral and physiologic effects of d-amphetamine is being studied. A small dose of amphetamine (0.3 mgm/kg) is administered to normal volunteer twins after medical and psychiatric screening. Preliminary data suggests stable and heritable responses in 1) behavioral ratings; 2) cortical evoked responses; 3) diurnal cycle of motor activity 4) neuroendocrine response, and; 5) pharmacokinetics of amphetamine. We do not find adrenergic receptor response to be stable and heritable. We have developed a new behavioral rating system to assist us in this work.

Other Investigators Continued:

Steven Targum, M.D.	Section on Psychogenetics	BPB NIMH
Linda Kessler, M.D.	Food and Drug Administration	BPB NIMH
Juliet Guroff, M.S.	Research Assistant	BPB NIMH

Project Description:

Pharmacogenetic studies in psychopharmacology have largely been devoted to inherited differences in drug metabolism as opposed to genetics of physiologic and psychologic responses to drugs. The behavioral pharmacogenetics of psychoactive drugs in particular is an area in which little systematic investigation has been undertaken, although there have been enough clinical reports of patients and relatives treated with the same medication to suggest that further study will be fruitful.

Our first pharmacogenetic protocol is a study of d-amphetamine. Individual differences in response to amphetamine have been used as a predictor of clinical response to tricyclic anti-depressants and as such may be an important clue to the genetic vulnerability to affective disorders. In this project, monozygotic and dizygotic twins and same-sex sibling pairs of volunteers are being studied. All persons with pre-existing psychiatric or medical disorders that are not minor are excluded.

d-Amphetamine is an indirect agonist of dopamine and norepinephrine neurons in the central nervous system. There has been much recent interest in the effects of amphetamine and amphetamine-like compounds such as methylphenidate in mania, schizophrenia and depression.

Amphetamine may produce mood changes of euphoria or dysphoria. Preliminiary results on four identical twin pairs show behavioral and mood responses to amphetamine are stable within individuals and are significantly heritable.

Intraindividual stability and heritability is also suggested in our results on cortical evoked potentials and serum growth hormone, prolactin, and cortisol.

Serial determinations of plasma amphetamine suggest a stable and heritable pharmacokinetic pattern for twin pairs. Activity measures, using the patient activity monitor (PAM) suggest characteristic changes in diurnal activity cycle with amphetamine.

Our data do not show consistent changes in norepinephrine or cyclic AMP.

A behavioral rating system with high interrater reliability has been developed. The system is illustrated with an audio-visual teaching tape. Tapes of the infusions are being retained for research purposes. A systematic interview technique for pharmacologic studies is also being developed.

Proposed Course of Project

40 more twins will be included in the study for a total of 13 identical pairs and 12 fraternal pairs. This project will proceed for at least one more year. The results will be correlated with results from another study on amphetamine response in bipolar patients before and after haloperidol.

Significance:

Heritable biologic defects in the affective disorders have not yet been discovered. There is evidence that drug responses can distinguish affectively ill people from normals, as well as distinguish sub-populations of affective disorder. If it can be demonstrated that these responses have heritable characteristics, they could be biologic markers for mood disorder determinable in the well state. This would be of considerable use in tracing vulnerability to mood disorder through families, and in genetic counseling of family members.

Our behavioral rating system and interview techniques will be helpful in standardizing results of studies in behavioral pharmacology.

Publications:

None.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00086-02 BP

PERIOD COVERED
October 1, 1977 through September 30, 1978

TITLE OF PROJECT (80 characters or less)

Outpatient Clinic for Genetic and Pharmacologic Studies of Affective Disorders

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	E. S. Gershon, M.D.	Chief, Section on Psychogenetics	BPB NIMH
OTHER:	L. R. Kessler, M.D.	Medical Officer	FDA
	E. D. Dibble, D.S.W.	Social Worker	BPB NIMH
	F. K. Goodwin, M.D.	Chief	CPB NIMH
	P. Gold	Chief, Outpatient & Follow-up Unit	CPB NIMH
	Y. B. Davenport, M.S.W.	Social Worker	CPB NIMH
	J. Nurnberger, M.D.	Sr. Staff Fellow	BPB NIMH
	N. Sitaram, M.D.	Research Psychiatrist	BPB NIMH
	J. C. Gillin, M.D.	Chief, Unit on Sleep Studies	BPB NIMH
	E. Frank, M.S.		Univ. of Pittsburgh

COOPERATING UNITS (if any)
CPB
CC Nursing
Univ. of Pittsburgh

LAB/BRANCH
Biological Psychiatry Branch

SECTION
Section on Psychogenetics

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.75	1.25	0.5

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The outpatient clinic provides a "well state" patient population for genetic and pharmacologic studies of persons with Unipolar (major depressive) and Bipolar (manic-depressive) disorders. Such a "well state" population is crucial in identifying biochemical, pharmacologic, or physiologic abnormalities which are present in persons vulnerable to affective disorder, and which may serve as genetic markers to affective illness. Preliminary results suggest that Bipolar patients in the well state have greater sensitivity to a cholinergic agonist (Arecoline) than normal controls.

PHS-6040
(Rev. 10-76)

Project Description:

The purpose of the project is to have a "well state" patient population who would be available for participation in the following research areas:

1. Identification of biochemical, pharmacologic, or physiologic abnormalities which are present in persons who have had major affective disorder but who do not currently require hospitalization.

2. Identification of biological markers of a genetically transmitted predisposition to affective disorders.

3. Creation of a pool of available patients and relatives for further protocols which will be developed and submitted on the genetics of affective disorders, and on longitudinal studies of affective disorders.

Patients are admitted to the study upon referral from the wards of the NIMH intramural program or from various community referral resources. Fifty-two patients (25 Bipolar I, 11 Bipolar II, 12 Unipolar, 4 others), are currently enrolled in the BPB portion of the outpatient clinic.

Identification of biochemical, pharmacologic or physiologic vulnerabilities to affective illness: The strategy in this type of study is to identify a biologic vulnerability to illness which is not state dependent but present or evocable even when the patient is in the "well state" or in remission.

Pharmacologic studies: Arecoline is a muscarinic cholinergic agonist which induces rapid eye movement (REM) sleep when given in sufficient dosage. This effect is blocked by scopolamine, which suggests that the effect is produced by stimulation of a central cholinergic receptor. In collaboration with the Sleep Studies Unit of the Biological Psychiatry Branch, we have given low doses of Arecoline intravenously during non-REM sleep to five Bipolar patients who were euthymic and off medications. At a dose of 1.0 mg., a significantly shortened time to the next REM period was produced in these patients as compared to several normal controls. Daytime infusion of the same dose produced no significant mood or behavior effects. This preliminary finding suggests increased sensitivity to cholinergic stimulation in the well state in Bipolar manic depressive illness.

Another project which is being administered in the outpatient clinic is the recently proposed protocol "Response to amphetamine infusion before and after chronic haloperidol treatment in Bipolar patients". A demonstration of supersensitivity of catecholamine receptors during a brief amphetamine infusion will be attempted in 20 patients with Bipolar manic-depressive illness in remission. Supersensitivity will be induced by two week treatment with clinical doses of haloperidol, a catecholamine receptor blocking agent used in Bipolar illness. Supersensitivity of CNS catecholamine receptors has not been previously demonstrated in man, although it has been proposed that this is a mechanism in the vulnerability to Bipolar illness. Behavioral, neuroendocrine, biochemical and neurophysiological responses to amphetamine infusion at two times will

be compared: (1) prior to haloperidol administration, and (2) after cessation of a two week course of haloperidol, during the time when supersensitivity has been present in basic pharmacologic studies.

Concurrently, the protocol "Pharmacogenetic studies of d-amphetamine in normal volunteers" is being carried out to study the inheritance of variability in the behavioral and physiologic effects of d-amphetamine, and to provide control data. The heritability of behavioral and biologic response will also be determined from the response parameters in members of a sibling or twin pair (see project report #Z01 MH 00085-02 BP).

Biological markers of a genetically transmitted predisposition to affective disorders. Linkage to known chromosomal markers is a strategy for demonstrating that a characteristic is inherited and for demonstrating the mode of inheritance. Patients and relatives in this clinic are studied for chromosomal linkage markers, such as blood group phenotype, HLA phenotype and known biochemical polymorphisms, as described in project report #Z01 MH 00084-04 BP.

Clinical studies of the well state in affective disorders: Pre-existing conceptions on familial and genetic questions in affective illness, and expectations of affective illness and its consequences in one's self and relatives, are being studied through questionnaire and clinical interviews. This information may prove useful for genetic counseling in psychiatry, a service for which there is a growing demand. A controlled study of marital adjustment using the KDS-15 questionnaire developed by E. Frank (Univ. of Pittsburgh) will be performed to continue studies of the disordered marital relationships that characterize Bipolar affective illness.

Significance of the Study: Identification of a biologic vulnerability detectable in the well state in Bipolar manic depressive illness would be of great importance for clinical treatment and also for identifying persons who might become ill in the future, including relatives of patients.

Proposed Course of Project: Studies of CNS and peripheral sensitivity to agonists and antagonists of monoamine and cholinergic receptors will continue to have highest priority over the coming years.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00116-04 BP

PERIOD COVERED

October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Interactions of Neurotransmitters and Membrane Proteins at Bimolecular Membranes

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. S. Kafka	Physiologist	BP NIMH
Other:	R. Blumenthal	Visiting Scientist	LTB NCI

COOPERATING UNITS (if any)

LAB/BRANCH

Biological Psychiatry Branch

SECTION

Section on Biochemistry and Pharmacology

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Dopamine-β-hydroxylase, an enzyme catalyzing the conversion of dopamine to norepinephrine, is found in the membranes of adrenal medullary cell chromaffin vesicles. Dopamine-β-hydroxylase interacts with its substrate, dopamine, and calcium at the bimolecular lipid membrane to form channels through which ions pass. As the ion conductance increases linearly with the potential across the membrane, the potential itself does not open or close, i.e., "gate" the channels. To produce a conductance increase, the natural conformation of the enzyme is required, and norepinephrine, the product of the reaction catalyzed by the enzyme, cannot substitute for dopamine, its substrate. The experiments on bimolecular lipid membranes may provide a physical model for a biological action of dopamine-β-hydroxylase. Dopamine-β-hydroxylase in the membrane of the adrenal medullary chromaffin vesicle may combine with its substrate, dopamine, to catalyze the conversion of dopamine to norepinephrine and at the same time increase the permeability of the vesicle membrane to ions, thereby affecting synthesis, storage, or release of the vesicle catecholamines.

Project Description:

Objectives. To study interactions between neurotransmitters, hormones, membrane proteins, and bimolecular lipid membranes (BLM). The bimolecular lipid membranes separating two aqueous compartments may serve as a model for the cell plasma membrane separating interstitial fluid from cytoplasm or a model of an intracellular membrane separating the cytoplasm from a vesicle interior, e.g., a secretory vesicle membrane.

Methods Employed: A BLM is formed so as to separate two aqueous compartments containing buffer and ions. A pair of KCL-calomel electrodes, one in each compartment, is used to apply a potential across the membrane and to conduct current resulting from the potential. At zero applied potential, a diffusion potential across the membrane can be measured. The electrical conductance of the membrane can be calculated from Ohm's Law.

Dopamine-β-hydroxylase, an enzyme catalyzing the conversion of dopamine to norepinephrine is added to the aqueous compartments and allowed to interact with a bimolecular lipid membrane made from oxidized cholesterol.

Major Findings:

(1) Dopamine-β-hydroxylase, added to both sides of the bimolecular lipid membrane in the presence of calcium and dopamine, increases the conductance of ions through the membrane. (2) The conductance increase occurs in discrete steps of 0.4 - 0.7 nmho each and is non-selective. (3) The conductance increases as the square of the dopamine-β-hydroxylase concentration. (4) Dopamine-β-hydroxylase, denatured by heat, does not increase the conductance. (5) When norepinephrine replaces dopamine, no conductance increase is observed. (6) The conductance increase is linear with the applied potential. (7) Dopamine-β-hydroxylase, added to only one side of the bimolecular lipid membrane, also increases the electrical conductance in the presence of calcium and dopamine. (8) When dopamine-β-hydroxylase is added to only one side of the membrane, the conductance increases linearly with the dopamine-β-hydroxylase concentration.

Significance to Biomedical Research and the Program of the Institute:

Dopamine-β-hydroxylase, an enzyme catalyzing the conversion of dopamine to norepinephrine, is found in the membranes of adrenal medullary cell chromaffin vesicles. Dopamine-β-hydroxylase interacts with its substrate, dopamine, and calcium at the bimolecular lipid membrane, to form channels through which ions pass. As the ion conductance increases linearly with the potential, the potential itself does not open or close, i.e., "gate," the channels. To produce a conductance increase, the natural conformation of the enzyme is required and norepinephrine, the product of the enzyme reactions, cannot substitute for dopamine.

The experiments on bimolecular lipid membranes may provide a physical model for a biological action of dopamine-β-hydroxylase. Dopamine-β-hydroxylase

in the membrane of the adrenal medullary chromaffin vesicle may combine with its substrate dopamine to catalyze the conversion of dopamine to norepinephrine and at the same time increase the permeability of the vesicle membranes to ions, thereby affecting synthesis, storage, or release of the vesicular catecholamines.

Proposed Course of the Project:

It is anticipated that this study will be completed within the period of this report.

Publications:

Kafka, M.S., Blumenthal, R., Walker, G.A., and Pollard, H.B.: Dopamine-β-hydroxylase changes bimolecular lipid membrane electrical conductance. Membrane Biochemistry, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00117-03 BP

PERIOD COVERED

October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

α- and β-Adrenergic and Prostaglandin Receptors in Human Blood Elements

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Marian S. Kafka	Physiologist	BP NIMH
	John F. Tallman	Biologist	BP NIMH
Other:	Craig C. Smith	Chemist	BP NIMH
	John W. Thomas	Chemist	BP NIMH

COOPERATING UNITS (if any)

Section on Psychobiology, BPB, NIMH; Section on Neuropsychopharmacology, BPB, NIMH; Laboratory of Clinical Psychopharmacology, NIMH; Clinical Psychobiology Branch, NIMH

LAB/BRANCH

Biological Psychiatry Branch

SECTION

Section on Biochemistry and Pharmacology

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	1.1	0.4

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Alpha- and β-adrenergic receptors and prostaglandin receptors are being studied in human blood cell preparations. The ability of adrenergic drugs to activate or inhibit adenylyl cyclase is used as a measure of their function. Binding of tritiated adrenergic receptor antagonists to membrane receptors on the blood cells is being measured. These correlative measures are being used to assess α- and β-adrenergic physiological function in normal human beings, patients with various psychiatric disorders, and patients receiving different psychopharmacologic agents.

PHS-6040
(Rev. 10-76)

Objectives:

(1) To develop methods to study the physiological function of α- and β-adrenergic receptors in easily obtainable human tissue. (2) To study alterations in receptor number or function in pathological conditions which are characterized by altered receptor sensitivity and in psychiatric diseases. (3) To provide an experimental model for the study of drug and/or physiologically induced changes in central receptor sensitivity in man. (4) To understand the membrane perturbations or alterations connecting receptors occupied by their agonists with the inhibition or activation of adenylyl cyclases and subsequent physiological events.

Methods Employed:

To measure α-adrenergic receptors, human platelets are prepared from freshly drawn blood. A portion of the platelets is washed and resuspended for measurement of cyclic AMP production. A second portion is homogenized to yield a membrane preparation which is washed and resuspended for the measurement of tritiated α-adrenergic antagonist binding. To measure β-adrenergic receptors a leukocyte preparation is made from human blood using a dextran polymer to induce rapid settling of the erythrocytes. The leukocyte-rich plasma is centrifuged at low speed to yield a leukocyte pellet. This pellet is washed several times in 0.9% saline solution and is used to measure binding of a tritiated β-antagonist and production of cAMP.

Cyclic nucleotides are determined after incubation of intact leukocytes or platelets with the drugs under study by a competitive protein binding assay for cAMP or by radioimmunoassay for cGMP.

Major Findings:

α-Adrenergic Function. Human platelets contain α-adrenergic receptors and prostaglandin-stimulable adenylyl cyclase, but not β-adrenergic receptors. Prostaglandin E_1 (PGE_1) stimulates the formation of cAMP in platelets and α-adrenergic agonists inhibit PGE_1-stimulated cAMP production in the order of potency, l-epinephrine $\geq$ l-norepinephrine >> l-isoproterenol. α-Adrenergic agonist inhibition of cAMP production is reversed by the α-adrenergic antagonists phentolamine and dihydroergocryptine, but not by β-adrenergic antagonists dl-propranolol or l-alprenolol. [^{3}H]dihydroergocryptine, an α-adrenergic antagonist, binds to human platelet membranes. Binding is inhibited by α-adrenergic agonists in the order l-epinephrine $\geq$ l-norepinephrine >> l-isoproterenol, and by α-adrenergic antagonists phentolamine, phenoxybenzamine, and dihydroergocryptine, but not by β-adrenergic antagonists dl-propranolol or l-alprenolol.

α-Adrenergic agonists inhibit cAMP production half-maximally at about the same concentration that they half-maximally inhibit binding of [^{3}H]dihydroergocryptine. The prostaglandin receptor in human platelets is stimulated by prostaglandins in the order: $PGE_1 > PGE_2 > PGF_{1\alpha} > PGF_{2\alpha}$.

β-Adrenergic Function. Leukocytes contain β-adrenergic binding sites and β-adrenergic and prostaglandin-stimulable adenylyl cyclases. The β-adrenergic-stimulable cyclase is particularly enriched in the lymphocyte-monocyte subfraction. The β-adrenergic receptor of leukocytes is stimulated by agonists in the following order of potency: l-isoproterenol > l-epinephrine > l-norepinephrine. The prostaglandin receptor is stimulated by individual prostaglandins in the following order: $PGE_1 > PGE_2 > PGF_{1\alpha} > PGF_{2\alpha}$.

Significance to Biomedical Research and the Program of the Institute:

It is anticipated that results obtained from these experiments will be directly applicable to an assessment of receptor functioning in various human disease states and in monitoring the physiological effects of various drug treatments. If the peripheral α- and β-adrenergic receptors are related to or can serve as a model for central nervous system adrenergic receptors, valuable information may be obtained for the first time about the functioning of these central receptors in psychiatric diseases.

Proposed Course:

Further examination of the mechanism of information transfer from α- and β-adrenergic and the prostaglandin receptor to adenylyl cyclase is anticipated. Presently, studies of these receptors are underway in schizophrenic patients, in patients with affective illness, hypertensive patients, and other patients receiving various therapeutic regimens.

Studies of β-adrenergic function are being carried out in schizophrenic patients who are receiving high doses of propranolol, a β-adrenergic blocker which is said to be of therapeutic value in schizophrenia. The blocking effects of this drug and possible supersensitivity following withdrawal from propranolol are being investigated.

Publications:

Kafka, M.S., Tallman, J.F., Smith, C.C., and Costa, J.L.: Alpha-adrenergic receptors on human platelets. Life Sci. 21: 1429-1438, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00118-02 BP

PERIOD COVERED

October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Induction of β-adrenergic Receptors in HeLa Cells

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	John F. Tallman	Research Biologist	BP NIMH
Others:	Richard C. Henneberry	Senior Scientist	LMB NINCDS
	Craig C. Smith	Chemist	BP NIMH
	Ayub Yousufi	Visiting Fellow	BP NIMH

COOPERATING UNITS (if any)

Laboratory of Molecular Biology, NINCDS

LAB/BRANCH

Biological Psychiatry Branch

SECTION

Section on Biochemistry and Pharmacology

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.5	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The induction of β-adrenergic receptors in HeLa cells has been studied using 1-[^{3}H]dihydroalprenolol to measure binding to the receptor and l-isoproterenol activation of adenylate cyclase to measure function. After sodium butyrate treatment, the number of 1-[^{3}H]dihydroalprenolol binding sites but not their affinity increases and the ability of l-isoproterenol to activate adenylate cyclase in butyrate treated cells is enhanced. The increase in binding sites and their "coupling" to adenylate cyclase requires the synthesis of new protein; the induction of receptors is specific for butyrate as other closely related short-chain fatty acids are less potent inducers. At low butyrate concentrations, it is possible to increase the number of receptors without increasing their ability to activate adenylate cyclase. By switching the cells to media with high butyrate, it is now possible to increase the ability of the receptors to activate adenylate cyclase. Thus, the increase in receptors and the increase in "coupling" are separate events.

PHS-6040
(Rev. 10-76)

The presence of β-adrenergic receptors in HeLa cells has been established using 1-[^{3}H]dihydroalprenolol to measure binding to the receptor and l-isoproterenol stimulated cAMP production to measure the ability of the receptors to function. Binding to these receptors is stereoselective, reversible, and rapid enough to account for the known properties of β-adrenergic receptors. The response to the presence of l-isoproterenol is blocked by classical β-adrenergic blockers but not α-adrenergic blocking drugs.

After sodium butyrate treatment, the number of binding sites for 1-[^{3}H]-dihydroalprenolol increases although the affinity of these receptors for 1-[^{3}H]dihydroalprenolol is unchanged. This increase in binding occurs in conjunction with an increase in the ability of the receptors to activate adenylate cyclase and enhance cAMP production in intact cells after treatment with l-isoproterenol. These increases are rapid, and noticeable increases in binding and cyclase activation occur within a few hours; the increase is virtually complete after 24 hours. It is possible to block the increase in both binding and adenylate cyclase activation with cycloheximide, an inhibitor of protein synthesis; Actinomycin D blocks the increase in binding sites but enhances activation of cyclase by β-adrenergic receptors at low concentrations. The effects of butyrate (a four-carbon fatty acid) on HeLa are rather specific as only propionic (C-3) and valeric (C-5) are able to mimic the effects of butyrate.

By manipulating the concentration of butyrate, it is possible to obtain almost complete induction of binding sites at low butyrate concentrations and by increasing the concentration of butyrate to effect coupling of preexisting receptors to adenylate cyclase. Thus the induction of receptors and coupling to adenylate cyclase seem to exist as separate events.

The effects of various compounds which have known actions on cell membranes and the motility of proteins on the cell surface have been examined either on both processes or by using a design which allows induction of binding sites and coupling to be examined separately. As an example, Concanavalin A inhibited induction of both β-adrenergic binding and coupling to adenylate cyclase by high butyrate concentration while it stimulated the coupling of pre-induced receptors to cyclase. This type of experimental design will allow us to sort out the role of membrane fluidity and "clustering" of receptors in their ability to activate adenylate cyclase.

The effects of guanyl nucleotides on the coupling of β-adrenergic receptors and adenylate cyclase has been studied in untreated and butyrate treated HeLa cells. In homogenates there is no requirement for GTP in the action of l-isoproterenol activation of adenylate cyclase. However, in membrane preparations from HeLa cells GTP is required for the activation of the adenylate cyclase. Although required in the membranes, GTP will not activate untreated cells to the level of activity present in butyrate treated cells.

Significance to Biomedical Research and the Program of the Institute:

The advantages of a model system to study receptor dynamics and function are tremendous compared to studies with animals. As cells in culture which are

readily manipulated provide a stable source of cells which respond in a consistent fashion, the effects of drugs at known concentrations at the cell surface may be studied. The examination of drug effects in the model system can lead to predictions of *in vivo* effects of these drugs in patients. It is also possible to study subsensitivity in this model system which may be related to clinical subsensitive states.

Proposed Course:

Research is currently underway into the following questions:

1. What is the mechanism of catecholamine-induced subsensitivity?

2. What is the nature of the second event which occurs after treatment of the HeLa cells with high concentrations of butyrate: Is there a membrane-bound factor whose synthesis is necessary for efficient coupling to occur?

3. Can we develop receptor- or butyrate-resistant mutants?

4. What is the role of membrane fluidity in these processes?

Publications:

Tallman, J.F., Fishman, P.H., and Henneberry, R.C.: Determination of sialidase activities in HeLa cells using ganglioside specifically labeled in N-acetyl-neuroaminic acid. Arch. Biochem. Biophys. 182: 556-562, 1977.

Henneberry, R.C., Smith, C.C., and Tallman, J.F.: Relationship between β-adrenergic receptors and adenylate cyclase in HeLa cells. Nature, 268: 252-254, 1977.

Tallman, J.F., Smith, C.C., and Henneberry, R.C.: Properties of β-adrenergic receptors in untreated and butyrate treated HeLa cells. Biochim. Biophys. Acta, in press, 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00119-02 BP

PERIOD COVERED

October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Putative Neurotransmitters and Modulators in Cerebrospinal Fluid

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	John F. Tallman	Biologist	BP NIMH
Other:	Candace B. Pert	Pharmacologist	BP NIMH
	Pauline Lerner	Chemist	BP NIMH
	Craig C. Smith	Chemist	BP NIMH
	John W. Thomas	Chemist	BP NIMH
	Haruo Shibuya	Visiting Fellow	BP NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Biological Psychiatry Branch

SECTION

Section on Biochemistry and Pharmacology

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.9	1.4	1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Methods were developed for the determination of cAMP, cGMP, γ-aminobutyric acid, substance P and endogenous opiate ligands. Using these methods, it is possible to measure these compounds in a variety of tissues and under many physiological conditions. In particular, all of the above compounds have been or are being measured in cerebrospinal fluid from human subjects. With the available cerebrospinal fluid from patients under study in the Sections of Psychobiology and Neuropsychopharmacology, no differences between patients with schizophrenia and affective disorders could be demonstrated for cAMP, cGMP and GABA.

PHS-6040
(Rev. 10-76)

Using radioimmuno, radioreceptor, or radioenzymatic methods it was possible to develop assays for cAMP, cGMP, GABA, substance P and endogenous opiate ligands. cAMP was measured by a protein binding method which utilized protein kinase from bovine adrenal cortex. The cAMP in the sample to be determined competes with radioactive cAMP for a limited number of binding sites on protein kinase. By using varying amounts of unlabeled cAMP, it is possible to construct a standard curve and thus quantitate the amount of cAMP in the sample. Similarly, GABA and endogenous opiate levels were determined by measuring the ability of the sample (or known amount of GABA or endogenous opiate) to displace the radioactive ligand from a brain membrane preparation which binds to GABA or opiates specifically and with a high affinity. Substance P and cGMP were measured using a radioimmunoassay with antibodies either prepared by us or purchased.

We have put a large effort into developing antibodies against β-endorphin to use the antibodies in developing an assay for β-endorphin which can be used on clinical samples. The assay at present will detect 0.05 pmol β-endorphin/sample. This is almost good enough to detect the endogenous levels of this compound in body fluids such as plasma or CSF. Efforts will continue to improve this antibody or extraction procedure until a successful assay is obtained. At present our assay is capable of determining the levels of β-endorphin after infusions.

Significance to Biomedical Research and the Program of the Institute:

Measurement of transmitters and modulators of brain function in cerebrospinal fluid may be important for understanding the metabolism and turnover of these compounds in brain. If differences exist between groups of patients, it may be possible to implicate a transmitter or biochemical event as being important in affective disorders or schizophrenia.

Proposed Course:

We are presently preparing the GABA study for publication and are about ready to measure substance P in a number of clinical samples. Attempts to develop a good valid assay for endogenous opiates and β-endorphins are continuing.

Publications:

None.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00120-01 BP

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

GABA-Benzodiazepine Interactions

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Dorothy W. Gallager	Research Neuropharmacologist	BP NIMH
	John F. Tallman	Biologist	BP NIMH
Other:	John W. Thomas	Chemist	BP NIMH

COOPERATING UNITS (if any)

LAB/BRANCH
Biological Psychiatry Branch

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Section on Biochemistry and Pharmacology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.2	0.7	0.5

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Interactions of γ-aminobutyric acid (GABA) with benzodiazepines have been studied electrophysiologically and biochemically in rats. Benzodiazepines enhance GABA inhibition of firing in the dorsal raphe nucleus but are inactive by themselves. Agents such as amino-oxyacetic acid (AOAA) which raise GABA levels in vivo lead to a potentiation of benzodiazepine inhibition. These effects are blocked by (+)bicuculline and picrotoxin. Pretreatment of animals with AOAA and muscimol (a GABA agonist) in vivo lead to enhanced binding of [^{3}H]diazepam measured in vitro. This enhanced binding is due to enhanced affinity of a specific benzodiazepine binding site in brain but no change in the number of sites. Including GABA or muscimol in direct [^{3}H]diazepam binding assays in vitro also leads to enhanced binding. This effect is stereospecifically blocked by bicuculline and provides direct evidence for a GABA-benzodiazepam interaction.

The effects of benzodiazepines (BZs) after both direct and systemic application were tested on central neuronal activity in intact rats using microiontophoretic and single-cell recording techniques. Based on evidence that the dorsal raphe nucleus has specific and independent receptors for 5HT, GABA and glycine, alterations in the firing rate of DR neurons following the administration of BZs were evaluated to determine whether the changes were the result of a direct interaction with 5HT receptors or due to interactions of BZ with GABA and/or glycine. Although the BZs did not alter the spontaneous firing rate of cells in the raphe nucleus, both the systemic and iontophoretic administration of these drugs were found to specifically potentiate the inhibitory response produced by GABA. No interaction with 5HT or glycine was observed. Since effects of the BZs on spontaneous neuronal activity in the raphe only follow pretreatment with AOAA (with increased levels of GABA) or muscimol (a GABA agonist), the data suggests that BZs may only have pronounced effects when GABAergic input is prominent.

Biochemical correlates of the electrophysiological effects were studied by investigating [^{3}H]diazepam binding. [^{3}H]Diazepam binds in rat brain cortex to sites which fulfill many of the criteria which should be fulfilled if these sites are pharmacologically relevant. Binding is rapid, reversible and of high affinity. The number of binding sites is limited and their distribution most closely parallels GABA binding.

When animals are pretreated with drugs such as AOAA or muscimol, enhanced [^{3}H]diazepam binding *in vitro* is found in brain. This enhancement is due to an increase in the affinity of the specific [^{3}H]diazepam binding site for its ligand. No change is demonstrated in the total number of sites. Drugs such as lioresol and GBL, which also activate GABAergic mechanisms, will enhance *in vitro* binding.

This effect can be replicated using an *in vitro* experiment with washed membranes from rat cortex. When GABA or muscimol are included in a [^{3}H]diazepam binding assay, a dose-dependent enhanced binding of [^{3}H]diazepam can be demonstrated. This effect is due to a change in the affinity of this binding site, not the total number of sites. The effect is antagonized by (+)bicuculline, an active GABA antagonist, and is less potently inhibited by (-)bicuculline, its less active stereoisomer.

Significance to Biomedical Research and Institute Program:

The mechanism of action of the benzodiazepines is largely unknown in spite of their wide use for their anxiolytic, muscle relaxant and anticonvulsant properties. Our results show that at least one mechanism of action may be their synergistic action with the GABAergic system, the major inhibitory transmitter in brain. Thus, these studies may lead to the elucidation of the functioning of this system and also to a more complete understanding of the mode of action of one of the most widely prescribed drugs in the world.

Proposed Course:

Continued studies into the actual interaction between these systems is expected. We also hope to purify one or more of the proteins involved with an eye to the reconstruction of an active system.

Publications:

Gallager, D.W.: Benzodiazepines: potentiation of a GABA inhibitory response in the dorsal raphe nucleus. Eur. J. Pharmacol., in press.

Gallager, D.W., Thomas, J.W., and Tallman, J.F.: Effect of GABAergic drugs on benzodiazepine binding site sensitivity in rat cerebral cortex. Biochem. Pharmacol., in press.

Tallman, J.F., Thomas, J.W., and Gallager, D.W.: GABAergic modulation of benzodiazepine binding site sensitivity. Nature, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00121-01 BP

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Dopamine-β-hydroxylase in Cerebrospinal Fluid

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Pauline Lerner	Chemist	BP NIMH
Other:	James C. Ballenger	Clinical Associate	BP NIMH
	L. Frank Major	Clinical Associate	CN NIMH
	Charles R. Lake	Clinical Associate	LCS NIMH
	Adrian Williams	Clinical Associate	ET NINCDS
	Robert M. Post	Chief, Section on Psychobiology	BP NIMH
	Frederick K. Goodwin	Chief	CP NIMH

COOPERATING UNITS (if any)
Clinical Neuropharmacology Branch, NIMH; Laboratory of Clinical Science, NIMH; Experimental Therapeutics Branch, NINCDS; Clinical Psychobiology Branch, NIMH

LAB/BRANCH
Biological Psychiatry Branch

SECTION
Section on Biochemistry and Pharmacology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.6	0.6	0.0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Dopamine-β-hydroxylase (DBH) and the compound it synthesizes, norepinephrine, have been measured in cerebrospinal fluid (CSF) from psychiatric patients and normal volunteers. The mean DBH values for the different groups--unipolar depressives, bipolar depressives, schizophrenics/schizoaffectives, alcoholics, personality disorders, and normal volunteers--were not significantly different. There was a significant positive correlation between DBH and norepinephrine in unmedicated depressed patients and in depressed patients treated with monoamine oxidase inhibitors. DBH and norepinephrine were not significantly correlated in CSF from alcoholics or normal volunteers.

PHS-6040
(Rev. 10-76)

Objectives:

Dopamine-β-hydroxylase and norepinephrine are released from storage vesicles when noradrenergic neurons fire. Dopamine-β-hydroxylase is being measured in human cerebrospinal fluid as a possible indicator of central noradrenergic activity. The relationship between cerebrospinal fluid dopamine-β-hydroxylase and cerebrospinal fluid norepinephrine is also being studied.

Methods:

A previously reported radioenzymatic assay for dopamine-β-hydroxylase has been modified to achieve the necessary sensitivity to measure reliably the very low dopamine-β-hydroxylase activity in cerebrospinal fluid. Norepinephrine is also measured by a radioenzymatic assay.

Major Findings:

There were no significant differences among the mean dopamine-β-hydroxylase levels for subjects from different diagnostic groups--affective illness, schizophrenia, alcoholics, personality disorders, and normal volunteers. However, some of these groups differed in the relationship between dopamine-β-hydroxylase and norepinephrine in the cerebrospinal fluid. In alcoholics and normal volunteers, there was no significant correlation between dopamine-β-hydroxylase and norepinephrine in cerebrospinal fluid. In contrast, cerebrospinal fluid dopamine-β-hydroxylase and cerebrospinal fluid norepinephrine were strongly correlated in unmedicated depressed patients. When depressed patients were treated with monoamine oxidase inhibitors (clorgyline and pargyline), there was also a significant correlation between norepinephrine and dopamine-β-hydroxylase, but the norepinephrine:dopamine-β-hydroxylase ratio was increased. The observations on depressed patients are consistent with the drug-induced accumulation of norepinephrine and the concomitant release of dopamine-β-hydroxylase and norepinephrine from noradrenergic nerves during firing. The monoamine oxidase inhibitors also caused a significant decrease in cerebrospinal fluid dopamine-β-hydroxylase. This decrease probably reflects a slowing of central noradrenergic firing, as has been shown in animal studies.

Significance to Biomedical Research and Institute Program:

These studies show that intra-individual changes in cerebrospinal fluid dopamine-β-hydroxylase activity may be useful as a marker of central noradrenergic activity.

Proposed Course of Project:

Dopamine-β-hydroxylase will be studied in cerebrospinal fluid from psychiatric patients treated with other drugs which affect the central noradrenergic system. Laboratory animals will also be used to study the effects of pharmacological treatments on dopamine-β-hydroxylase activity in cerebrospinal fluid.

Publications:

Lerner, P., Goodwin, F.K., van Kammen, D.P., Post, R.M., Major, L.F., Ballenger, J.C., and Lovenberg, W.: Dopamine-β-hydroxylase in the cerebrospinal fluid of psychiatric patients. Biol. Psychiatry, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00122-01 BP

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Urinary 3-Methoxy-4-hydroxy-phenylethylglycol in Psychiatric Patients

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Pauline Lerner	Chemist	BP NIMH
Other:	Robert M. Post	Chief, Section on Psychobiology	BP NIMH
	James C. Ballenger	Clinical Associate	BP NIMH

COOPERATING UNITS (if any)

LAB/BRANCH
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INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.4	0.4	0.0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

3-Methoxy-4-hydroxy-phenylethylglycol (MHPG) is a major metabolite of norepinephrine which can be measured accurately in urine samples by a gas chromatographic assay. MHPG has been measured in urine from unmedicated and drug-treated depressed patients. The unmedicated patients had urinary MHPG levels similar to those cited in the literature. Neither clonidine nor tegretol had a significant effect on the amount of MHPG in the patients' urine.

PHS-6040
(Rev. 10-76)

Objectives:

This study was undertaken in order to establish baseline levels of urinary 3-methoxy-4-hydroxy-phenylethylglycol (MHPG) in psychiatric patients of different diagnostic groups and normal volunteers and to determine the effect of drug treatment on urinary MHPG.

Methods:

Urinary MHPG was measured using a gas chromatograph equipped with an electron-capture detector. The analytical method was validated by comparison studies with standard samples tested by researchers in several different laboratories.

Major Findings:

The baseline MHPG values for the depressed patients were in close agreement with those reported by other groups. The effect of clonidine, an α-adrenergic agonist, on urinary MHPG was studied in four patients. Three of the patients showed no significant change, and the fourth had a significant decrease in urinary MHPG. The latter patient had the highest pre-treatment MHPG and also received the largest dose of clonidine. A study of 13 patients on tegretol showed that this drug did not induce a significant change in urinary MHPG.

Significance to Biomedical Research and Institute Program:

The amount of MHPG in urine may give an index of the turnover rate of norepinephrine, a neurotransmitter reportedly involved in affective illness.

Proposed Course of Project:

MHPG will be measured in urine from other depressed patients and normal volunteers.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00123-01 BP

PERIOD COVERED

October 1, 1977 to September 1, 1978

TITLE OF PROJECT (80 characters or less)

Blockade by Chronic Lithium Treatment of Presynaptic Dopamine Supersensitivity

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Dorothy W. Gallager	Research Neuropharmacologist	BP NIMH
Other:	William E. Bunney, Jr.	Chief	BP NIMH
	Agu Pert	Psychologist	BP NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Biological Psychiatry Branch

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Section on Biochemistry and Pharmacology

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.7	0.5	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Using microiontophoretic and single-cell recording techniques, the effect of chronic lithium (Li) treatment on the development of supersensitivity to dopamine (DA) was investigated on DA-containing cells in the zona compacta region of the substantia nigra in the intact rat. Supersensitivity, as measured by increased sensitivity to iontophoretically applied DA and to the i.v. administration of graded doses of the DA agonist apomorphine, was observed in presynaptic (DA-containing) cells following chronic haloperidol (HAL) administration. Animals treated chronically with both Li and HAL failed to show any increase in sensitivity to either iontophoretically applied DA or to i.v. apomorphine.

PHS-6040
(Rev. 10-76)

Objectives:

To determine whether, at the level of individual catecholamine neurons in the central nervous system, chronic lithium treatment could affect the development of supersensitivity.

Methods Employed:

Microiontophoresis and extracellular unit recording techniques. Radioactive tracer experiments also used to determine disposition of [^{3}H]haloperidol.

Major Findings:

The iontophoretic techniques provide a direct demonstration that the sensitivity of dopamine-containing (presynaptic) cells can be increased by chronic neuroleptic (haloperidol) administration. This has previously been demonstrated only postsynaptically (cells receiving dopamine input) in the caudate. In addition, chronic lithium treatment prevents the increase in sensitivity of dopamine-containing neurons to dopamine and dopamine agonists.

Significance to Biomedical Research:

Alterations in catecholamine receptor sensitivity have been theorized to be a factor in the etiology of affective disorders. Since lithium therapy has been shown to be effective in preventing recurrent episodes of mania and depression in manic-depressive illness, the blockade of presynaptic supersensitivity development at the level of individual dopamine neurons by chronic lithium treatment may have clinical relevance.

Proposed Course of Project:

The effects of chronic lithium treatment on the sensitivity of pre- and postsynaptic neurons in other central nervous system transmitter systems will be investigated.

Publications:

Gallager, D.W., and Pert, A.: Afferents to brain stem nuclei (brain stem raphe, nucleus reticularis pontis caudalis and nucleus gigantocellularis) in the rat as demonstrated by microiontophoretically applied horseradish peroxidase. Brain Res. 144: 257-275, 1978.

Gallager, D.W., Pert, A. and Bunney, W.E. Jr.: Haloperidol-induced presynaptic dopamine supersensitivity: blockade by chronic lithium. Nature, 273: 309-312, 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00124-01 BP

PERIOD COVERED

October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Mechanism of Action of Lithium in the Treatment of Affective Disorders

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Agu Pert	Psychologist	BP NIMH
Other:	Jack E. Rosenblatt	Clinical Associate	BP NIMH
	Candace B. Pert	Pharmacologist	BP NIMH
	John F. Tallman	Biologist	BP NIMH
	William E. Bunney, Jr.	Chief	BP NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Biological Psychiatry Branch

SECTION

Section on Biochemistry and Pharmacology

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.8	1.3	1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Long-term treatment of rats with haloperidol produced an increased sensitivity to the locomotor and stereotypic effects of apomorphine. This behavioral dopaminergic supersensitivity was also accompanied by increased [^{3}H]spiroperidol binding in the striatum. However, rats treated concurrently with lithium and haloperidol failed to develop both behavioral sensitivity to apomorphine and increased striatal dopamine receptor binding. While chronic lithium had no effect on dopamine binding, it appeared to increase α-adrenergic binding and decrease β-adrenergic binding. It is suggested that the ability of lithium to prevent recurrent manic-depressive episodes may be related, in part, to these effects on the catecholamine receptors.

PHS-6040
(Rev. 10-76)

Changes in catecholamine receptor sensitivity have been postulated to underlie a number of pathologies in man. Tardive dyskinesia may follow or be intensified by the termination of chronic treatment with antipsychotic neuroleptic drugs. The development of tardive dyskinesia has been assumed to be the result of increased sensitivity of dopaminergic receptors. Oscillations in catecholamine receptor sensitivity have also been proposed to be a factor in the etiology of affective disorders especially manic-depressive illness (Bunney and Post, 1977). Since lithium therapy is effective in alleviating and preventing the recurrence of manic and manic-depressive symptomatology, it was of special interest to determine whether chronic lithium treatment would alter the development of catecholamine receptor supersensitivity.

Effect of Lithium on Development of Behavioral Supersensitivity. Rats were treated for 21 days with either haloperidol (1 mg/kg), haloperidol and lithium, saline or saline and lithium. Lithium was administered in the diet and produced plasma levels that are equivalent to therapeutic levels in humans (0.8 to 1.0 meq/l). Seven to eight days after the cessation of drug treatments all locomotor activity and stereotypy were assessed following the injection of apomorphine. A similar study was performed in which supersensitivity was induced by chronic treatment with the tyrosine hydroxylase inhibitor α-methyl-p-tyrosine (100 mg/kg for 21 days). To study the effects of lithium on the development of presynaptic dopamine receptor supersensitivity, four groups of rats were treated as above. Seven days after the termination of treatments, all animals were tested in activity chambers after administration of 0.05 mg/kg apomorphine--a dose that has been shown to depress activity due to its predominant actions on presynaptic dopamine receptors.

Effect of Lithium on Binding of [^{3}H]Spiroperidol after Chronic Treatment with Haloperidol. Supersensitivity induced by chronically administered neuroleptics as well as nigrostriatal lesions has recently been found to be accompanied by an increase in striatal dopamine receptor sites. This series of studies was initiated to ascertain whether lithium treatment would inhibit the increase in dopamine receptor binding induced by chronic dopaminergic blockade.

Four groups of rats were treated as above in the behavioral studies. Seven days after discontinuation of all treatments, rats were sacrificed and their caudates dissected. The freshly dissected caudates were homogenized and washed in Tris buffer. The final suspension was incubated in triplicate at 37°C with tritiated spiroperidol and either (+) or (-)butaclamol.

Effect of Lithium on Changes in α- and β-Adrenergic Sensitivity. Chronic treatment with tricyclic antidepressants has been found to produce β-adrenergic receptor subsensitivity and α-adrenergic receptor supersensitivity. This series of studies examined the effects of lithium on changes in α- and β-receptor sensitivity following chronic administration of tricyclic antidepressants and lesioning of adrenergic neurons with 6-hydroxydopamine.

Major Findings:

Apomorphine induced locomotor activity and stereotyped behavior were increased in haloperidol pretreated rats but not in rats that had been pretreated with both haloperidol and lithium. Lithium also prevented the development of supersensitivity to amphetamine following chronic inhibition of tyrosine hydroxylase with AMPT. In addition to preventing post-synaptic supersensitivity, lithium also appeared to inhibit the development of pre-synaptic supersensitivity to low doses of apomorphine.

Specific binding of [^{3}H]spiroperidol was increased in caudates of haloperidol pretreated rats but not in rats that had been pretreated with both lithium and haloperidol. Lithium treatment alone did not affect striatal [^{3}H]spiroperidol binding. Lithium also did not affect levels of haloperidol in whole brain or in the caudate.

Chronic administration of imipramine was found to induce β-adrenergic receptor subsensitivity but had no effect on α-receptor binding. Lithium did not affect the β-receptor subsensitivity induced by imipramine. Surprisingly, chronic lithium by itself induced β-receptor subsensitivity and α-receptor supersensitivity. Furthermore, both α- and β-receptor supersensitivity induced by intraventricular 6-OHDA were inhibited by lithium. Thus, the actions of lithium on the catecholamine systems appear to be quite complex. While lithium appears to stabilize the dopamine receptor, it also seems to increase the sensitivity of the α-adrenergic system and decrease the sensitivity of the β-adrenergic system.

Significance to Mental Health Research and the Program of the Institute:

Lithium is one of the most widely used and efficacious compounds in the treatment of manic and manic-depressive illness. Understanding its mechanism of action will aid in defining the factors underlying the expression of mania and manic-depressive illness.

Proposed Course:

1. Attempts will be made to analyze the effects of chronic lithium on other neurotransmitter systems.

2. The effects of lithium will be assessed on the development of sub- and supersensitivity in the cholinergic, serotonergic, GABAergic and enkephalinergic systems.

Publications:

Pert, A., Rosenblatt, J.E., Sivit, C., Pert, C.B., and Bunney, W.E. Jr.: Long-term treatment with lithium prevents the development of dopamine receptor supersensitivity. Science, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00125-02 BP

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Chemotactic Peptide Receptors

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Candace B. Pert	Pharmacologist	BP NIMH
	Elliott Schiffmann	Biochemist	LDBA NIDR

COOPERATING UNITS (if any)
Laboratory of Developmental Biology and Anomalies, National Institute of Dental Research

LAB/BRANCH
Biological Psychiatry Branch

SECTION
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INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.0	0.0	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Project has been discontinued.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00143-03 BP

PERIOD COVERED

October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Physiological Function of Opiate Receptors and Their Endogenous Ligands

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Candace B. Pert	Pharmacologist	BP NIMH
Other:	Donald L. Bowie	Chemist	BP NIMH
	Haruo Shibuya	Visiting Fellow	BP NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Biological Psychiatry Branch

SECTION

Section on Biochemistry and Pharmacology

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.8	1.8	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Opiate receptors are also receptors for naturally occurring peptides called endorphins or enkephalins. Experiments in this laboratory are aimed at clarifying the identity and function of these peptides.

PHS-6040
(Rev. 10-76)

Objectives:

1. To determine the chemical and environmental stimuli which release β-endorphin from the pituitary into the blood.

2. To gain a better understanding of opiate receptor function.

Methods Employed:

1. Radioimmunoassay of plasma β-endorphin with antiserum raised against albumin-coupled derivatives.

2. Receptor binding assay of radiolabeled alkaloid and peptide opiates to rat membranes.

Major Findings:

1. Opiate receptors in rat brain are normally about 50% occupied by enkephalin and/or endorphin. Behavioral manipulation of rats can alter the extent of opiate receptor occupancy.

2. It is possible to measure plasma β-endorphin levels utilizing adsorption and elution methodology.

3. Obesity in rats and mice appears to be associated with increased levels of β-endorphin in the pituitary gland and increased levels of circulating β-endorphin in the plasma. Naloxone specifically reverses overeating in obese rodents.

Significance to Biomedical Research and Institute Program:

Opiates produce profound mood-altering, sleep-inducing and analgetic effects when administered to humans. These effects are due to an artificial mimicking of the natural effects of endogenous morphine-like factor(s) on their specific membrane opiate receptors. This new, previously undescribed neuronal and hormonal system, is a likely candidate for regulating mood, sleep, pain suppression, and urinary output--four behavioral effects which exhibit pathological changes in mental illness. Detailed study of the biochemical functioning and brain distribution of "morphinergic neurons" may elucidate their role in normal and abnormal behavioral states.

Proposed Course:

We are currently employing the β-endorphin assay in clinical samples obtained (1) from all hours during a 24-hour cycle, (2) from opiate addicts and their controls, (3) from patients suffering from various forms of mental illness, (4) after extensive jogging, and (5) after imbibing various psychoactive drugs. We are assaying rat levels in these and other situations. We plan future experiments on how closely obesity is coupled to β-endorphin, including examining whether lesions of the lateral hypothalamus which promote overeating can have their effects blocked by maintaining rats on a naltrexone diet.

Publications:

Pert, C.B., Bowie, D.L., Pert, A., Morell, J.L., and Gross, E.: Agonist-antagonist properties of N-allyl-[D-Ala]^2-met-enkephalin. Nature, 269: 73-75, 1977.

Pert, C.B.: A request for serum samples from psychiatric patients with associated autoimmune disease: is some psychosis caused by an autoimmune response to neurotransmitter receptors? Communications in Psychopharmacology, 1: 307-309, 1977.

Pert, C.B., and Bowie, D.L. Behavioral Manipulation of Rats Causes Alterations in Opiate Receptor Occupancy. In Usdin, E., Bunney, W.E. Jr. and Kline, N.S. (Eds.) Endorphins in Mental Health Research. London, Macmillan Press, 1978, in press.

LaMotte, C.C., Snowman, A., Pert, C.B., and Snyder, S.H.: Opiate receptor binding in Rhesus monkey brain: association with limbic structures. Brain Res., in press.

Pert, C.B., and Gulley, B.L.: The Mechanism of Opiate Agonist and Antagonist Action. In O'Malley, B.W. and Birnbaumer, L. (Eds.) Hormone Receptors, Vol. II Peptide Hormones. New York, Academic Press, in press.

Pert, C.B.: Opiate receptors and pain pathways. NRP Bull., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00147-03 BP

PERIOD COVERED

October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Behavioral and Physiological Effects of Morphine-like Brain Peptides

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Agu Pert Psychologist BP NIMH

Other: Louise DeWald Psychologist BP NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Biological Psychiatry Branch

SECTION

Section on Biochemistry and Pharmacology

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.3	0.8	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Microinjections of opiates into various brain structures revealed that these compounds exert differential effects on motor behaviors depending on site of injection. Opiates and neuroleptics were found to produce catalepsy through different brain structures. Opiates appear to activate the dopaminergic nigro-striatal pathways through some indirect mechanism since iontophoretic applications of morphine to zona compacta neurons failed to influence their spontaneous activity. Opiates appear to produce at least part of their analgesic actions by inhibiting the transmission of pain information through the nucleus giganto-cellularis. Stress-induced analgesia does not appear to be mediated by the release of enkephalin.

PHS-6040
(Rev. 10-76)

Objectives:

The objectives of this project were to define the mechanisms and sites of action of enkephalins in brain and to evaluate possible physiological functions for these novel peptides.

Effects of Opiates and Opioid Peptides on Motor Behaviors: Sites and Mechanisms of Action. In addition to their analgesic effects, opiates also have profound effects on motor behaviors. Small doses of morphine produce increases in spontaneous locomotor activity while large doses cause an initial depression and catatonia which is followed by motor excitation. The purpose of these studies was to analyze the mechanisms and sites of action of opiate alkaloids and opioid peptides in modulating motor behaviors. Rats were injected with morphine or D-Ala^2-met-enkephalin in various regions of the brain through chronic indwelling cannulae. Spontaneous motor activity and catatonia were assessed following injections.

To analyze the actions of opiates on the nigro-striatal system we employed the rotational model introduced by Ungerstedt (1971) and electrophysiological procedures. In the first series of studies we evaluated the effects of acute and chronic morphine on rotational behavior in animals that had been lesioned unilaterally in the substantia nigra with 6-OHDA. In the second series of studies we assessed rotational behavior following unilateral injections of opiates and opioid peptides into various components of the nigro-striatal system. In the third series we examined the effects of morphine on the spontaneous activity of neurons in the zona compacta of the substantia nigra using both single-cell and iontophoretic recording techniques.

Analgesic Mechanisms of Action of Morphine in the Brainstem. Using microinjection techniques we have previously demonstrated that the periaqueductal gray matter of the mesencephalon, as well as the nucleus gigantocellularis in the pontine reticular formation, are critical foci for mediating opiate analgesia. Interestingly both of these regions are high in both enkephalinergic terminals and opiate receptors. Lesion, as well as electrical brain stimulation techniques were used to assess the mechanisms of action of opiates in the brainstem and the function of enkephalinergic pathways to the pain processing system.

Analgesic Activity of Novel Neuropeptides. Substance P has been found to be high in the dorsal horn of the spinal cord, as well as in brain regions that are associated with the processing of pain information. This peptide has been postulated to be an excitatory neurotransmitter of primary afferents that carry pain information. On the other hand, systemic, as well as intracerebral injections of substance P have been reported to produce analgesia in rats. We attempted to analyze the actions of substance P in the periaqueductal gray matter, an area that has been shown to be a focus of opiate analgesia.

Bombesin is another neuropeptide that has been found to be present in the mammalian brain. The effects of this peptide on nociception, as well as other behaviors were evaluated following intracerebral injections.

The Involvement of Enkephalinergic Systems in Stress-Induced Behavior. Madden et al. (Nature, 265:358, 1977) have suggested that enkephalin may be released in brain during stress. They found that foot-shock stress in rats increased levels of enkephalin in brain and concurrently elevated nociceptive thresholds. We attempted to evaluate the involvement of enkephalins in stress-induced analgesia (SIA) using pharmacological, as well as physiological techniques.

Major Findings:

Both opiates and opioid peptides were found to produce an initial depression of spontaneous motor activity which was followed by hyperactivity. Tissue injections revealed differential effects of opiates on motility depending on injection site. Injections into the nucleus accumbens and substantia nigra produced only hyperactivity without a depressant component. Injections into the lateral hypothalamus, ventral thalamus, dorsal hippocampus and periaqueductal gray matter produced only depression of motor behavior. Cataleptic effects of opiates also appeared to be mediated through the hippocampus and periaqueductal gray matter. Although some investigators have postulated that both opiates and neuroleptics induce catatonia by inhibiting dopaminergic transmission in the caudate nucleus, it became apparent from our studies that these two classes of compounds affect entirely different neural substrates. Chlorpromazine was found to produce catalepsy following injections into the caudate nucleus and was entirely ineffective after injections into the periaqueductal gray matter while morphine induced catatonia following injections into the periaqueductal gray matter and was ineffective in the caudate. Naloxone was also found to decrease locomotor behavior, indicating that some enkephalinergic pathways that modulate motor behaviors are tonically active.

Chronic administration of morphine was found to produce rotational behavior in animals that had been lesioned unilaterally in the substantia nigra with 6-hydroxydopamine. The rotational behavior increased with repeated injections and indicated that morphine was activating the dopaminergic nigro-striatal pathways since systemic haloperidol was effective in blocking morphine-induced rotations. Direct injections of morphine, enkephalin and β-endorphin into the substantia nigra induced profound rotational behavior contralateral to the injection indicating that the direct action of morphine in the substantia nigra activated the ascending dopaminergic pathways.

This activation, however, does not appear to be direct since we have not found morphine to influence the activity of dopaminergic neurons in the zona compacta of the substantia nigra. Systemic injections of morphine, on the other hand, do appear to increase the spontaneous activity of zona compacta neurons.

Direct injections of morphine into nucleus giganto cellularis (nGC) produced a slight increase in nociceptive thresholds and potentiated the analgesic effects of morphine. Electrical stimulation of the nGC failed to produce analgesia at low current intensities. At higher current levels rats exhibited profound motor reactions. Following stimulation the animals appeared to be somewhat unresponsive to nociceptive input. This apparent analgesia was not reversed by naloxone and appeared to be related more to disruption of motor

performance than to attenuation of noxious sensory input. The effect of morphine in the nGC appears to be related to the inhibition of pain information which is transferred through this relay.

Stress-induced analgesia was not found to be inhibited by naloxone, therefore ruling out the possibility that this phenomenon is mediated by the release of enkephalin by stress. We also failed to detect increases in opiate-like substances in the cerebrospinal fluid of the Rhesus monkey following electrical foot-shock. Stress-induced analgesia was, however, antagonized to some extent in hypophysectomized rats suggesting that at least part of the analgesic reaction to stress is mediated by some pituitary factor.

Substance P was found to produce analgesia following microinjections into the periaqueductal gray matter only in relatively high doses (30 µg). This "analgesia" was not antagonized significantly by naloxone. It does not appear that substance P produces analgesia through the enkephalinergic system. Bombesin also appeared to produce analgesia following injections into the periaqueductal gray matter. This effect was also not antagonized by naloxone.

Significance to Mental Health Research and the Program of Institute:

Since opiates are among the most potent psychotomimetic and euphoragenic compounds, it is of special significance that there exist endogenous compounds in brain which are apparently mimicked by opiates. There is substantial evidence that these compounds may be novel neurotransmitters or neuroregulators. If this is the case, then it is quite conceivable that such neurohumors may play an important role in affective states. Furthermore, dysfunctions in the neuronal systems encoded by these peptides may underlie some of the etiology of mental disorders and neurological dysfunctions.

Proposed Course:

1. Further attempts will be made to define environmental and physiological conditions that can modify enkephalin levels in brain.

2. More emphasis will be placed on the use of electrophysiological techniques to ascertain the effects of enkephalins on defined neuronal systems. Special emphasis will be directed at analyzing the effects of enkephalin on the mesolimbic and nigrostriatal dopamine systems.

Publications:

Chang, J.K., Shimizee, M., Fong, B.T.W., Peterson, W.J., Pert, C.B., and Pert, A.: ARG-B_h-Endorphin: a synthetic β-lipotropin fragment with potent analgesic activity in vivo. FEBS Letters, in press.

Pert, A.: Central Sites Involved in Opiate Actions. In Fishman, J. (Ed.): The Bases of Addiction. Berlin, Dahlem Konferenzen, 1978, in press.

Pert, A., DeWald, L.A., Liao, H., and Sivit, C.: Effects of Opiates and Opioid Peptides on Motor Behaviors: Sites and Mechanisms of Action. In Usdin, E., Bunney, W.E. Jr., and Kline, N.S. (Eds.) Endorphins in Mental Health Research. Hampshire, Macmillan Press, 1978, in press.

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Pert, C.B., Bowie, D.L., Pert, A., Morell, J.L., and Gross, E. Agonist-antagonist properties of N-allyl-[D-Ala]^2-met-enkephalin. Nature, 269: 73-75, 1977.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00155-01 BP

PERIOD COVERED

December 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Brain Neuropeptide Receptors

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Candace B. Pert	Pharmacologist	BP NIMH
Other:	Terry W. Moody	Chemist	DR NIDA
	Duncan P. Taylor	Postdoctoral Fellow	BP NIMH

COOPERATING UNITS (if any)

Division of Research, NIDA

LAB/BRANCH

Biological Psychiatry Branch

SECTION

Section on Biochemistry and Pharmacology

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.8	1.8	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Receptors in brain membranes which mediate the pharmacological effects of the neuroactive peptides bombesin and vasointestinal peptide have been demonstrated by direct binding. The regional and subcellular distributions of these peptide receptors suggest that these peptides are neurotransmitters which may mediate important brain functions.

Objectives:

To develop a method for demonstrating the hypothetical bombesin and vasointestinal peptide (VIP) receptors on rat brain membranes.

Method Employed:

Direct binding of radiolabeled peptides was developed using iodinated peptides, peptidase inhibitors, and rapid filtration technology.

Major Findings:

1. Rat brain contains about 10 fold less bombesin receptors than opiate and most other neurotransmitter receptors. The ability to produce hypoglycemia or hypothermia by a number of bombesin analogs is closely correlated with their ability to bind to the bombesin receptor indicating that it is through this receptor that they exert their pharmacological effects. The bombesin receptor is clustered on synaptic membranes and shows a marked heterogeneous regional distribution with the highest binding found to be in the hippocampus and the lowest binding in the pons medulla.

2. VIP receptors seem to closely follow the distribution of endogenous VIP. Receptors are highest in cortical areas, the hippocampus, hypothalamus, striatum and thalamus. Not a single behavioral experiment exists to correlate the binding to the VIP receptor with pharmacologic action. However, the receptor displays the same pharmacological specificity as the VIP receptor found in acinar cells in the pancreas.

Significance to Biomedical Research and the Program of Institute:

It is simplistic, but certainly the discovery of what components make up the brain represents a most important advance in understanding how the brain works. Characterization of the distribution of new putative peptide neurotransmitter receptors can lead to the rational design of behavioral and neurophysiological experiments aimed at elucidating neurotransmitter function.

Proposed Course:

1. We have preliminary evidence that the brain contains a bombesin-like peptide. It may be possible to isolate this peptide using the radioreceptor assay which we are currently developing. We plan to figure out which cortical neurons have which peptide neurotransmitters through a series of lesioning studies. Moreover, we plan to examine the behavioral effects of these peptides.

Publications:

Moody, T.W., Pert, C.B., Rivier, J., and Brown, M.R.: Bombesin: specific binding to rat brain membranes. Proc. Nat. Acad. Sci. USA, in press.

Aswanikumar, S., Schiffmann, E., Corcoran, B.A., Pert, C.B., Morell, J.L., and **Gross**, E.: Antibiotics and peptides with agonist and antagonist chemotactic activity. Biochem. Biophys. Res. Comm. 80: 464-471, 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00326-05 CN

PERIOD COVERED

October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Clinical neuropharmacology and psychobiology of mania and depression.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Dennis L. Murphy	Chief, Clin. Neuropharmacol. Branch	NIMH
OTHER:	Jonathan L. Costa	Staff Physician	CN NIMH
	Robert M. Cohen	Clinical Associate	CN NIMH
	Carol F. Hoover	Social Worker	CN NIMH
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	David Pickar	Clinical Associate	CN NIMH
	Stanley L. Slater	Clinical Associate	CN NIMH
COLLAB:	Monte S. Buchsbaum	Unit Chief	BP NIMH
	Edward F. Donnelly	Psychologist	SMR NIMH
	J. Christian Gillin	Research Psychiatrist	BP NIMH
	Daniel P. van Kammen	Unit Chief	BP NIMH
	Herbert Weingartner	Acting Chief	LPP NIMH

COOPERATING UNITS (if any)

Nursing Department, Unit 3-East
Clinical Center Rehabilitation

LAB/BRANCH

Clinical Neuropharmacology Branch

SECTION

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NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
7.5	4.5	3.0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In comparative studies of the behavioral and biological effects of two structurally-related monoamine oxidase (MAO) inhibitors, clorgyline was found to be a highly selective inhibitor of MAO type A during longer-term administration in man. Pargyline had greater inhibitory effects on MAO type B, but also partially inhibited MAO-A in the doses used. Preliminary behavioral data indicate greater antidepressant and antianxiety effects with clorgyline than pargyline, although both drugs had activating effects. In other studies, the effects of these drugs and of another MAO inhibiting antidepressant, tranylcypromine, on the diurnal secretion of plasma cortisol, growth hormone and prolactin and on the sleep EEG were examined in depressed patients.

Project Description:

Objectives: Individuals with depression, mania, and related disorders with affective components, including individuals with severe menstrual cycle-related psychopathology, are studied in attempts to understand the psychological and biological mechanisms involved in therapeutic drug effects in these disorders. As individual differences in psychoactive drug responsiveness appear to depend upon many psychological and biological factors, a variety of study approaches are utilized.

Methods Employed:

1. Behavioral and psychological assessment: Pretreatment evaluation of patients requires information from interviews of the patient and family, from psychometric approaches, from direct behavioral observation using various quantitative scales and from patients's self-ratings. The elucidation of individual and patient subgroup differences in drug response depends upon this information obtained by the clinical staff, including psychiatrists, psychologists, social workers and nursing personnel. Subsequent evaluation of drug response depends upon objective behavioral assessment as well as self-rated psychological change as obtained from a number of quantitative scales, several of which have been developed on this unit.

2. Biological assessment: Plasma, platelets, urine, and cerebrospinal fluid are collected for the measurement of enzymes, levels of biogenic amines and their metabolites, other chemical variables, and drug levels. Electrophysiologic measurement of sleep and of cortical evoked potentials are accomplished in collaborative studies with investigators in the Biological Psychiatry Branch.

Major Findings:

The series of studies outlined below represents work in varied stages of completion, as most clinical studies extend over a period of two to three years.

Evaluation of Clorgyline and Pargyline as Antidepressants and Inhibitors of Monoamine Oxidase (MAO) Types A and B in Man. This study, which is in its second year, is an attempt to define whether any greater therapeutic benefit (either greater efficacy or fewer side effects) might be obtained from clorgyline compared to pargyline. These two MAO-inhibitors are structurally similar propargylamine compounds, but manifest differential inhibitory potencies on the two forms of MAO in vitro. During four weeks of treatment with clorgyline, the selective MAO-A inhibitor, platelet monoamine oxidase (MAO) activity was unchanged. During a similar 4-week crossover treatment period with pargyline, the selective MAO-B inhibitor, platelet MAO activity was essentially completely inhibited in the same individuals. The differential effects of the two drugs on platelet MAO, which consists exclusively of the MAO-B form, suggests that the in vitro selectivity of clorgyline, and possibly of pargyline, on MAO-A and MAO-B may be maintained in vivo during long-term

administration in man. Reductions in blood pressure, heart rate and plasma amine oxidase activity were generally similar in magnitude during treatment with both drugs, however, suggesting that either these effects are non-specific consequences of both MAO-A and MAO-B inhibition or that pargyline also inhibited MAO-A activity.

The relatively higher pargyline doses used (approximately 90 mg/day, versus 30 mg/day for clorgyline) may be a factor in contributing to some lesser selectivity in pargyline's effects. The doses chosen for this study were based upon estimates from the animal literature and from in vitro studies of the amounts of each drug which would yield maximally selective effects, as well as on doses used in earlier clinical studies of these drugs. Insufficient information is available on the comparative metabolism and plasma levels obtained with these two drugs to estimate whether the doses chosen were fairly equivalent. Another factor, however, which may be more important is that under in vitro conditions, the selectivity of clorgyline for MAO-A versus B is over ten-fold higher than that of pargyline for MAO-B versus A.

The inhibition of plasma amine oxidase by both clorgyline and pargyline in vivo contrasted markedly with the failure of these drugs to act as inhibitors in vitro. There is some evidence that propargylamine and similar drugs can be metabolized in vivo to yield a compound active against non-flavin-linked amine oxidases including the plasma enzyme. We have previously noted a similar inhibition of human plasma amine oxidase activity in vivo but not in vitro during studies with the N-substituted cyclopropylamine, Lilly 51641, a very active MAO-A inhibitor.

Speculations that selective MAO-A or MAO-B inhibition might yield more effective antidepressant effects and/or fewer side effects have not previously been assessed in a single patient group randomly assigned to MAO-A and MAO-B inhibitors. Limited, non-comparative data indicate that clorgyline and, to some extent, pargyline have some antidepressant effects, a conclusion concordant with preliminary information from our behavioral data on the initial patients included in this study.

This data from the Hamilton Depression Scale, the Brief Psychiatric Rating Scale and several self-rating-scales suggest that while both drugs had some activating effects, more frequent and more marked antidepressant and antianxiety effects occurred during the four week treatment period with clorgyline than with pargyline.

Neuroendocrine Effects of Antidepressant Drugs. In a study pursuing the possible mechanism for the elevation in plasma prolactin levels observed during treatment with pargyline and clorgyline, the effects of these two drugs and of another MAO inhibitor, tranylcypromine, on the diurnal secretion of plasma cortisol, growth hormone and prolactin and on the sleep EEG were studied in a group of depressed patients. Samples of venous blood were drawn via an indwelling catheter every 20 minutes for 24 hours during a pretreatment period and the drugs again during treatment with the sleep electroencephalogram was recorded between 11:00 and 7:00 A.M. on the same days that the blood

samples were drawn for hormonal assays. While the expected marked reductions in rapid eye movement sleep occurred during the drug treatment periods, the initial results from a small number of patients do not indicate a strong relationship between the sleep effects of the drugs and the prolactin changes. Further data analysis will evaluate possible correlations with clinical response and with the other hormone changes during treatment with the drugs.

Attempts to Predict Antidepressant Drug Responses By Biological and Psychological Measures. Acute activation and euphoriant responses to replicated single day trials with d-amphetamine proved to have moderately high correlations with subsequent antidepressant responses to imipramine and lithium carbonate during 3-4 weeks of treatment. These studies, initiated on 3-East when Dr. van Kammen was part of our group, indicate that common factors underly responsiveness to different drug groups in individual depressed patients. Preliminary data using MMPI sub-scales devised by Dr. Donnelly and Mr. Waldman suggest that predictive estimates of antidepressant responses to lithium and imipramine may also be obtained from pretreatment psychological tests.

Other Studies. Additional areas of investigation in patients with affective disorders include studies of personality features in subgroups of depressed patients, conducted in collaboration with Dr. Donnelly, and studies of memory and cognition in relation to mood state and drug effects, accomplished in collaboration with Dr. Weingartner. Studies of monoamine-related enzymes and monoamine metabolites in platelets, plasma, cerebrospinal fluid and urine from depressed patients are described in other project reports from our Branch.

Publications:

de la Vega, C.E., Slater, S., Ziegler, M.G., Lake, C.R., and Murphy, D.L.: Reduction in plasma norepinephrine during fenfluramine treatment. Clin. Pharmacol. Ther. 21:216-221, 1977.

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Slater, S.L., Lipper, S., Shiling, D.J., and Murphy, D.L.: Elevation of plasma-prolactin by monoamine-oxidase inhibitors. Lancet, 2:275-276, 1977.

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Donnelly, E.F., Goodwin, F.K., Waldman, I.N., and Murphy, D.L.: Prediction of antidepressant responses to lithium. Am. J. Psychiatry, 135:5, 1978.

Murphy, D.L., Campbell, I., and Costa, J.L.: The brain serotonergic system in the affective disorders. Prog. Neuro-Psychopharmacology, 2:1-31, 1978.

van Kammen, D.P., Marder, S.R., Murphy, D.L., and Bunney, W.E., Jr.: MAO activity, CSF amine metabolites, and drug-free remission in schizophrenia. Am. J. Psychiat. 135:567-569, 1978.

Murphy, D.L., Lipper, S., Slater, S., and Shiling, D.: Selectivity of clorgyline and pargyline as inhibitors of monoamine oxidases A and B in vivo in man. Psychopharmacology (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00327-06 CN

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Marital Conflict of Manic-Depressive Patients

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	C. F. Hoover	Social Worker	CN NIMH
OTHER:	R. G. Fitzgerald	Former NIMH Clinical Associate	CN NIMH
	J. J. Bartko	Mathematical Statistician	MB NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH
Clinical Neuropharmacology Branch

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.6	.5	.1

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This study compares manic-depressive patients, their spouses, and individuals from the community in terms of marital conflict, as revealed on the Conflict in Marriage Scale (CIMS).

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: Anger, hostility, and punitiveness, directed at the self and others, have long been seen as important dimensions in manic-depressive illness. The current research was undertaken (1) to examine self-reported levels of verbally expressed anger and dissension in the marriages of these patients and their spouses, compared to the acknowledged conflict of marital pairs in the community; (2) to attempt a discrimination between manic-depressive subjects, spouses, and community subjects on the basis of conflict item responses; (3) to compare patients, spouses, and community pairs on dominance and dependence, and (4) to explore similarity or complementarity of partners on issues of marital conflict and decision making.

Methods Employed:

An agree-disagree card sort, the Conflict in Marriage Scale (CIMS) was administered to 42 manic-depressive patients and their spouses, as well as to 30 comparable marital pairs from the local community. A discriminant function analysis was performed in an effort to discriminate patients, spouses, and community individuals on the basis of their responses to 67 items of the CIMS. In addition, subject responses indicating anger were assessed in relation to coping mechanisms reported, to produce a "conflict ratio" for each individual and couple. These ratios were then used for the purpose of comparing levels of verbally-expressed conflict in the marriages.

An additional 11 manic-depressive patients and their spouses have now been tested. A further three couples who are the parents of manic-depressive offspring have been added to an accumulating sample of these parents for future comparison with the parents of schizophrenics, normals, and maladjusted patients.

Major Findings:

1. Manic-depressive patients and their spouses reported significantly more conflict than community "well" pairs.
2. Patients reported significantly higher levels of verbally expressed marital conflict than their spouses did.
3. Based upon their responses to 67 items of the CIMS, patients, spouses, and community individuals could be distinguished at a high level of probability if sex were known in advance.
4. Spouses were reported to make family decisions more often than either manic-depressive patients or same-sexed community individuals.
5. Conflict ratios between husband and wife in manic-depressive marriages showed virtually no correlation, although the conflict ratios of community pairs were significantly correlated. This suggests that, on dimensions of marital conflict, complementarity may be a greater factor than similarity in manic-depressive marriages.
6. Patients were less likely than their spouses to get busy with work and activity as a means of dealing with anger.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH00328-03 CN

PERIOD COVERED

October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Monoamine Oxidase Activity and Behavior

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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OTHER:	Jonathan L. Costa	Staff Physician	CN NIMH
	Iain C. Campbell	Visiting Fellow	CN NIMH
	Leslie F. Major	Clinical Associate	CN NIMH
COLLAB:	Monte S. Buchsbaum	Unit Chief	BP NIMH
	Donald E. Redmond, Jr.	Assoc. Professor, Psychiatry, Yale Univ.	
	Elliot S. Gershon	Section Chief	BP NIMH
	Richard J. Wyatt	Director	DSMR NIMH
	Robert Coursey	Asst. Professor, Psychology, Univ. of MD	
	Paul Grof	Professor, Psychiatry, McMaster Univ., Canada	

COOPERATING UNITS (if any)

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Yale University, Department of Psychiatry

LAB/BRANCH

Clinical Neuropharmacology Branch

SECTION

INSTITUTE AND LOCATION

ADAMHA, NIH, NIMH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.3	2.0	2.3

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project is evaluating the associations between individual differences in platelet monoamine oxidase activity and human behavior. Biochemical studies of different human, monkey and rodent tissues and of individual cell lines grown in tissue cultures have added further information that monoamine oxidase types A and B are separate enzymes. Attempts are also being made to find and characterize more selective inhibitors of each MAO enzyme type. Studies of psychiatric, neurologic and other patient groups continue to indicate associations between reduced MAO activity and psychopathology in patients and their families. Similarly, studies in student populations have found moderately strong tendencies for certain personality features (such as sensation-seeking characteristics) and vulnerability to psychosocial difficulties (including suicide) to be associated with reduced MAO activity.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: This project investigates the apparent relationships between monoamine oxidase (MAO) activity and behavior. Because individual differences in MAO activity and drug effects on MAO activity are most readily studied in man using blood platelets as sources of the enzyme, the project has had as a primary objective the more complete biochemical characterization of platelet MAO activity in comparison to MAO activity in brain, liver and other tissues, including cell lines from tissue culture preparations. The study of factors which affect platelet MAO activity and which may be relevant to the interpretation of differences in MAO activity found in psychiatric patient subgroups is another primary objective.

Methods Employed:

Tissues obtained for the study of monoamine oxidase characteristics include human and other mammalian platelets, brain, heart, liver and kidney. Dr. Redmond has provided platelets and other tissues from vervet and rhesus monkeys as well as several other non-human primate species. Dr. Richelson has provided tissue culture preparations of C6 rat glial cells and mouse neuroblastoma cells. Dr. Wyatt has provided platelet samples from chronic schizophrenic patients, Dr. van Kammen samples from acute schizophrenic patients, Dr. Grof samples from a manic-depressive group in Canada, and Drs. Buchsbaum and Haier samples from normals. A large number of studies correlating MAO activity with clinical, biological (e.g., urinary MHPG, CSF metabolites, plasma norepinephrine and DBH) and pharmacological responses are being conducted on the 3-East Unit and collaboratively with other NIMH-IR investigators. Several different MAO assay procedures have been adapted or developed in this laboratory for the study of MAO-A and -B activities in platelets and other tissues. Techniques for the study of enzyme activity in different platelet populations have been worked out by Drs. Costa and Corash. Dr. Campbell has initiated investigations of the effects of chronic administration of selective inhibitors of the MAO A and B forms on neurotransmitter amines, amine metabolism and receptor function.

Major Findings:

Laboratory Studies of Monoamine Oxidase A and B Forms.

While most tissues in most species manifest both MAO-A and -B activities, our work demonstrated that two cell lines grown in tissue culture, the mouse neuroblastoma N1E-115 line and the C6 glial line, apparently possess only MAO-A activity, and the human platelet only MAO-B activity. These tissues have now been used to examine the effects of another group of MAO inhibitors, N-substituted cyclopropylamines, which like the propargylamines exhibit some selective effects on the two enzyme forms. A range of selectivity has been found associated with structural modifications on the side chain, and two drugs with selective MAO-B inhibitory properties (L-54761 and L-54748) identified. These drugs produced greater inhibition of the platelet enzyme than the neuroblastoma enzyme (in contrast to the effects of the MAO-selective

inhibitor in this series, L-51641) and, as expected, were less effective inhibitors of serotonin than phenylethylamine oxidation (again in contrast to L-51641). The availability of these drugs and a large number of related compounds having both side chain and ring modifications may be of value in studies of the active sites of the two enzyme forms, in studies of the physiological consequences of selective MAO inhibition, and possibly for clinical studies, as L-51641 has been the subject of several pilot investigations in man.

In further studies of the distribution of monoamine oxidase A and B forms, MAO-B activity has been found in non-human primate platelets whereas platelets from other species appear to have either no MAO activity (rodents) or predominantly MAO-A activity (the rabbit). Non-human primate brain also possesses predominantly MAO-B activity in contrast to the rat, which has a majority of MAO-A activity. Brain regions of vervet and rhesus monkeys were found to vary in the relative amounts of MAO-B and MAO-A activity. A study evaluating the correlations between MAO-A and MAO-B activities in brain, liver, platelet and other tissues in the vervet monkey is underway. Plasma amine oxidase activity in the rhesus monkey was found to be influenced by sex but not age differences, and to be at least partially dependent upon genetic factors.

Reduced Monoamine Oxidase Activity as a Vulnerability Factor for Psychopathology.

We have continued to explore the question of whether reduced monoamine oxidase activity might comprise a biological vulnerability factor for psychopathology. In a large majority of reports, chronic schizophrenic patients and patients with bipolar affective disorders and with alcoholism have been found to manifest reductions in platelet MAO activity. Some other disorders of childhood recently examined including primary autism and Gilles de la Tourette's syndrome are not associated with platelet MAO activity changes. The reductions in the major psychiatric disorders are not explained by drugs or other known illness-related factors. Platelet MAO activity is a generally stable characteristic of the individual, and several twin and sibling studies have shown that genetic differences account for most of the inter-individual differences in activity. Identical twins discordant for schizophrenia and first-degree, well relatives of bipolar patients and alcoholic patients also show equivalent reductions in MAO activity, suggesting that this difference represents a familial factor and is not the result of illness. Recent preliminary data suggest, however, that reduced MAO activity in family members of bipolar patients does not occur in parallel with illness in these families.

MAO-inhibiting drugs produce psychomotor activating effects in man and animals, and the use of these drugs clinically is associated with significant behavioral toxicity. MAO-inhibiting drugs markedly potentiate the biological and behavioral effects of many monoamines and their precursors. MAO-inhibition would thus seem to render the organism more vulnerable to a variety of enogenous agents and stressors.

In explorations of the hypothesis of reduced platelet MAO activity as a vulnerability factor or as an index of such a factor, psychological test differences and the occurrence of psychosocial difficulties in students and behavioral differences in rhesus monkeys have been found to be associated with MAO activity differences.

Interactions of reduced MAO activity with psychophysiological factors such as cortical evoked response measures have been found to be associated with increased suicide risk in both patient and non-patient populations. It thus seems possible that vulnerability to psychopathology may be related to the consequences of genetic (or, in some cases, non-genetic) differences in MAO activity along the lines of models provided by some other clinical disorders such as glucose 6-phosphate dehydrogenase deficiency, gout, diabetes mellitus and porphyria.

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Schooler, C., Zahn, T.P., Murphy, D.L., and Buchsbaum, M.S.: Psychological correlates of monoamine oxidase activity in normals. J. Nerv. Ment. Dis. 166:177-186, 1978.

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Murphy, D.L., Baulu, J., and Redmond, D.E., Jr.: Plasma amine oxidase activity in 136 normal rhesus monkeys: Relationships between enzyme activity and age, sex and genetic factors. J. Med. Primatol. 6:360-366, 1978.

Murphy, D.L., Donnelly, C.H., Richelson, E., and Fuller, R.W.: N-substituted cyclopropylamines as inhibitors of MAO A and B forms. Biochem. Pharmacol. (in press).

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Murphy, D.L.: Substrate-selective monoamine oxidases: Inhibitor, tissue, species and functional differences. Biochem. Pharmacol. (in press).

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00329-03 CN

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Platelets as Models for the Study of Neurotransmitter Function

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J.L. Costa	Staff Physician	CN NIMH
OTHER:	D.L. Murphy	Chief	CN NIMH
COLLAB:	K.L. Kirk	Chemist	LC NIAMDD
	L. Corash	Staff Physician	CP CC
	E.S. Silbergeld	Staff Associate	ETB NINCDS
	J. Nutt	Clinical Associate	ETB NINCDS
	K.D. Pettigrew	Statistician	TSM NIMH
	M.A. Smith	Medical Student, UCSD, San Diego, CA.	
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COOPERATING UNITS (if any)
UCSD Medical School, San Diego, CA: Dept. of Chemistry, Harvard Univ., Cambridge, MA: Bell Labs., Murray Hill, NJ: Thrombosis Research Center, Temple Univ., Phil., PA: Naval Blood Research Lab., Boston, MA.

LAB/BRANCH
Clinical Neuropharmacology Branch

SECTION

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	1.2	1.3

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☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Amine storage, metabolism, uptake, and release are examined in platelets with a variety of techniques including radiolabelled tracers, nuclear-magnetic resonance and microanalytical electron microscopy. The studies have four principal objectives: (1) to delineate the factors controlling the intracellular disposition of serotonin and other biogenic amines; (2) to evaluate the role of psychoactive drugs, genetically-linked disorders, physiologic aging, and species differences on the dynamics of platelet aminergic function; (3) to correlate predictive factors such as monoamine oxidase activity in human-platelet sonicates with the in vitro function of intact platelets; (4) to use the concepts and techniques developed in connection with the study of platelets to evaluate structure-function relationships in synaptosomes.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: This work extends our investigation of factors which influence or determine the ability of platelets to accumulate, metabolize, sequester, and release biogenic amines. Many of the techniques employed reveal that the uptake and intracellular movement of amines proceeds in a structured but complicated fashion. Our studies are directed toward providing a detailed understanding of the molecular basis for the observed phenomena. In addition, we are beginning to lay the groundwork for understanding the limits within which these processes may change in response to genetic and environmental factors, such as in different species, in patients with the Chediak-Higashi syndrome, Duchenne's syndrome, Huntington's disease, in patients receiving long-term treatment with lithium and other drugs, and during platelet senescence in vivo and in vitro. Concomitantly, some of the insights and techniques resulting from work with platelets have been employed to study synaptosomal function.

Methods Employed:

1. General Preparative Procedures. Platelets are isolated from a variety of species and from patients with genetic disorders or those receiving long-term drug therapy. Cells may be stored under controlled conditions (with Drs. Valeri and Vecchione) or separated by density-gradient centrifugation into fractions of differing physiologic ages (with Dr. Corash). Synaptosomes and isolated brain mitochondria are prepared from homogenates of rat caudate or rat whole brain by subfractionation through sucrose gradients or with mannitol (with Dr. Silbergeld).

2. Examination of Amine Fluxes in Intact Platelets. Total platelet serotonin (5HT) content, both immediately following isolation and following in vitro incubation with exogenous 5HT, is measured by an enzymatic assay. C^{14}- and H^3-labelled amines, including H^3-labelled 6-fluoroserotonin and 4,6-difluoroserotonin, are used to follow the uptake of amines into platelet cytoplasm and vesicles, as well as the release and metabolism of these substances.

3. Nuclear-Magnetic-Resonance Studies. Large numbers of platelets are isolated by plateletphoresis. The nuclear magnetic resonance of phosphorous residues associated with both stored and metabolic nucleotides and phosphates are examined in resting cells, following secretion, and following treatment with metabolic poisons (in cooperation with Drs. Dobson, Ugurbil, and Shulman). The disposition of fluorinated compounds sequestered in storage sites or cytoplasm is studied by F^{19}-NMR of cells prelabelled with 4,6-difluoroserotonin, 2-fluoroadenosine, or 2-fluoro-2-deoxyglucose (with Drs. Dobson, Valeri, and Vecchione).

4. Morphological Electron-Microscopic Studies. Whole mounts and thin sections of platelets, synaptosomes and brain mitochondria are examined by both transmission electron microscopy and scanning-transmission electron microscopy. Changes in the number and appearance of platelet storage vesicles and mitochondrial dense granules can thus be assessed in normal profiles, following a variety of treatments, and in cells from different types of patients.

5. Analytical Electron-Microscopic Studies. Specific areas inside whole mounts of platelets, synaptosomes, and brain mitochondria can be analyzed by X-ray microanalysis and electron energy-loss spectroscopy. Since very small quantities of fluorine can be localized intracellularly with electron energy-loss spectroscopy. the compartmentation of a variety of fluorinated biogenic amines can be studied.

Major Findings:

1. Effects of Physiological Aging or Storage on Platelet Function. As human platelets age in the circulation or are stored for prolonged periods, they experience progressive loss of their storage vesicles, and their ability to transport extracellular 5HT across the plasma membrane decreases with time. In addition, platelet monoamine-oxidase activity decreases with increasing platelet age. Since the number of mitochondria per platelet appears to remain unchanged throughout the platelet lifespan, the enzyme molecule itself may possess a relatively short functional half-life. Nevertheless, this phenomenon does not appear to contribute significantly to the inter-individual variations observed when large patient populations are studied.

2. Effects of Species Differences and Genetic Disorders on Platelet Function. Rabbit and dog platelets, which store different amounts of 5HT and other amines than do human platelets, contain storage vesicles (dense bodies) with widely differing amounts of magnesium, calcium, and phosphorus. Taken together with our findings that the 5HT content of human-platelet vesicles can be varied over a wide range without a concomitant change in vesicular calcium and phosphorus, this data suggests that 5HT need not be stored intracellularly in a specific type of phosphorus-cation complex. Furthermore, dense-body numbers and calcium and phosphorus content can be dramatically altered in the Chediak-Higashi syndrome and Duchenne's muscular dystrophy. Amine uptake into platelet cytoplasm or vesicles, in contrast, appears less subject to great variability, since it proceeds essentially unchanged in platelets from patients with Huntington's disease, Duchenne dystrophy, or the Chediak-Higashi syndrome.

3. Evaluation of the Complexity of Amine Uptake, Storage, and Exchange. Amine uptake into platelets, which can be examined over very short time periods at physiologic concentrations (10^{-8}M), displays a number of properties which are at variance with those described previously. Although 5HT uptake requires the presence of extracellular sodium, it appears not to be co-transported with this cation. Counter-transport does not occur, and the uptake can proceed unchanged even when a considerable amount of 5HT is present in platelet cytoplasm and the vesicles are filled to their maximal capacity (see below). Once inside the cell, amines appear to move between compartments in a structured fashion: (1) 5HT newly added to the cytoplasm from the extracellular medium does not mix with 5HT added to the same compartment following its displacement or loss from vesicles. (2) Cytoplasmic 5HT newly added from the extracellular medium will enter vesicles only when these are not filled to capacity, and when the concentration of 5HT in the extracellular medium is negligible. Interestingly, although both 5HT and 6-fluoro-5HT in cytoplasm will enter vesicles, difluoro-5HT will not. (3) 5HT entering the cytoplasm following loss or displacement

from vesicles is not metabolized and does not re-enter vesicles, but moves instead to the extracellular medium. (4) 5HT in vesicles of platelets from normal volunteers represents only about 20% of the total amount which can be stored in this compartment (the maximal storage packet size for 5HT). New 5HT from the extracellular medium continues to enter this compartment even when vesicles are filled to capacity, and acts to cause movement of the 5HT already inside vesicles to the cytoplasmic compartment.

4. Spectroscopic Approaches to Platelet Function. The application of P^{31} and F^{19} nuclear-magnetic-resonance studies to intact platelets suggests that vesicular difluoro-5HT, and possibly adenine nucleotides, are about 4 orders of magnitude more restricted in motion than if they were free in solution. Cytoplasmic difluoro-5HT is also significantly less mobile than in free solution, suggesting that differential binding may account for some of the phenomena observed with radiolabelled-tracer studies. Using electron energy-loss spectroscopy, it has been possible to confirm the anatomical localization of vesicular difluoro-5HT to dense bodies. When cells are permitted to accumulate difluoro-5HT for relatively short periods (30 seconds), every vesicle in a given platelet appears to contain significant amounts of the fluorinated compound. In addition, analysis of the intracellular lithium distribution in platelets from patients receiving long-term lithium therapy suggests that the majority of the intracellular lithium is sequestered in dense bodies. Dense-body calcium and phosphorus content appears normal despite the presence of the lithium.

5. Evaluation of Synaptosomal Function Employing Microanalytical Techniques. Changes in the composition of mitochondrial dense granules in synaptosomes can be evaluated by X-ray microanalysis--a factor which may assist in evaluating the role of mitochondrial calcium accumulation in synaptosomal function. The toxic effects of lead poisoning may be related to alteration of mitochondrial calcium sequestration, since lead accumulates in the dense granules following even a brief exposure of synaptosomes to extracellular lead.

6. Evaluation of the Role of Monoamine Oxidase in Platelet Function. Although monoamine oxidase in intact human platelets appears to be capable of metabolizing biogenic amines at rates approximating those seen in platelet sonicates, this activity does not prevent the accumulation of these amines in either platelet cytoplasm or vesicles. Furthermore, in vivo or in vitro treatment with monoamine-oxidase inhibitors which completely abolishes metabolism of some amines fails to prevent the metabolism of other amines.

Significance to Biomedical Research and the Program of the Institute: These studies use platelets as models for understanding neurotransmitter function in three ways: (1) They focus on the extension of the techniques and models developed during the course of the studies to other amine-storing systems such as synaptosomes. (2) They seek to evaluate the spectrum of factors influencing platelet uptake, metabolism, storage, and release of amines, and to determine the physical and chemical basis for the observed behavior. (3) Using our improved understanding of platelet aminergic function, this work attempts to evaluate how the function may be altered by genetic and environmental factors, and to assess the relationship of observed alterations to the genesis and therapy of diseases which may involve disorders of aminergic function.

Publications:

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00446-09 CP

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)
Inpatient Clinical and Psychopharmacological Studies of Manic-Depressive Disorders

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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OTHER:	Thomas Wehr	Chief, Clinical Res. Unit	CP/NIMH
	Yolande Davenport	Chief, Unit on Family Studies	CP/NIMH
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	Athanasios Zis	Guest Worker	CP/NIMH
	Irl Extein	Clinical Associate	CP/NIMH
	Helena Calil	Guest Worker	CP/NIMH
	Robert Post	Chief, Clinical Res. Unit	BPB/NIMH
	Dennis Murphy	Chief	CN/NIMH

COOPERATING UNITS (if any)
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LAB/BRANCH
Clinical Psychobiology Branch

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.2	2.8	

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☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The overall objective of this integrated group of research studies is a more comprehensive understanding of the pathophysiology of manic-depressive disorders, particularly with regard to the complex interrelationships among clinical phenomenology, diagnosis, biological variables, and pharmacologic response. The studies are longitudinal; drug trials are double-blind. Major projects involve: (1) evaluation of tricyclic antidepressants which show specificity for a given monoamine neurotransmitter; (2) the study of phase shifts in sleep in cycling patients; (3) the manipulation of sleep as a possible treatment of depression; (4) the evaluation of pimozide, an experimental antipsychotic drug, as a relatively superior drug for "atypical" manias with schizoaffective features (5) the use of biological prediction of drug response including AER, MHPG, CSF, amine metabolites, neuroendocrine parameters and melatonin in blood and urine.

PHS-6040
(Rev. 10-76)

Names, Laboratory and institute affiliations, and titles of principal investigators and all other professional personnel engaged on the project continued:

Monte Buchsbaum	Chief, Unit on Percep. and Cog. Studies	BPB/NIMH
Christian Gillin	Acting Chief, Unit on Sleep Studies	BPB/NIMH
Edward Donnelly	Research Psychologist	St. E's/NIMH
Arthur Prange	Prof. of Psychiatry	U. of No. Carolina

Project Description: Z01 MH 00446-09 CP

This project description encompasses a wide range of research studies not covered in other individual project descriptions, many of which are continuations of ongoing investigations. In this group of studies the overall objective is a more comprehensive understanding of the pathophysiology of manic-depressive and schizo-affective disorders, particularly in relation to specificity of diagnosis, existence of clinically or biologically identifiable subgroups (including subgroups which may predict drug response), the nature of predisposing factors, and the interrelationship between pharmacological and psychodynamic factors in clinical improvement.

Work on the standardization of clinical methodology has continued. Use of the Research Diagnostic Criteria (RDC) (described in detail in the July 1976 - September 1977 report on Project Z01 MH 00446-08 CP) is now routine. Discharge review conferences are being used to establish both a final RDC diagnosis and assess the extent to which each patient has "typical endogenous" depressive episodes. In order to facilitate this task a subjective "typicality" scale is being assessed and an extended record of endogenous features being maintained.

In order to minimize uncontrolled environmental variables the ward milieu has been structured according to a group model. Formal group psychotherapy is scheduled twice a week with a therapist and the head nurse. The only changes in group membership involve admissions or discharges of patients. A primary nurse and psychiatrist work together with each patient to provide medical care, to assist in decision-making and to address concerns that arise. The goal of the ward structure is to provide the necessary optimal management for each patient. Although a comprehensive research program on the interaction of affective illness and the psychosocial milieu is not a part of the Branch's program, some specific questions in this area are addressed.

Behavioral data used in evaluating changes are collected on a longitudinal basis throughout the course of hospitalization using twice daily global ratings by a trained nursing research team; these ratings focus on depression, mania, psychosis, anger and anxiety. Twice a week a modified form of the Brief Psychiatric Rating Scale (BPRS) is scored for each patient. Information regarding families is obtained through ongoing contact between the social worker and the patient's relatives. Motor activity is recorded continuously as described in Project #Z01 MH 00450-04 CP.

Sleep is recorded at half-hour intervals throughout the night on all patients. Selected patients have been studied for sleep parameters utilizing all night EEG sleep recordings in collaboration with the central sleep facility of the DCBR (Dr. Gillin). This effort has expanded following findings during the last year that sleep phases may systematically change in cycling patients and that manipulation of the time of day when an individual sleeps may provide a form of treatment (see Z01 MH 00450-04 CP). Certain patients may have sleep EEG's taken routinely for as long as a month.

All drug trials are conducted under double-blind conditions with alternating active and placebo periods in a non-random design. The use of placebo substitution following active drug increases one's level of confidence in the efficacy of a compound in an individual patient. This strategy is important in the evaluation of drug effects because of the frequency of spontaneous remissions and exacerbations in manic-depressive illness.

The major areas of study and major findings during the last year can be divided into separate areas:

1. The ongoing effort towards refinement of instruments for behavioral assessment has continued. In collaboration with the other clinical units use of the Brief Psychiatric Rating Scale (BPRS) has been underway, with cross-ward evaluation of interrater reliability and further development of the modified correlations between individual items and/or clusters of items with the global scores for mania, depression and psychosis. One immediate practical application has been the ability to identify discrepant ratings and ascertain whether this is secondary to rater misunderstanding or error.

2. The main antidepressant drug trial underway on the ward involves a crossover between Zimelidine and desmethylimipramine, the former having highly selective effects on reuptake in brain serotonin systems, while the latter is highly specific for norepinephrine systems. The results of this crossover trial will be integrated with our amine metabolite findings which suggest the existence of amine specific "subgroups" of patients with major depressive disorder. Because Zimelidine was not clinically available until May of this year, all trials to date have used desmethylimipramine.

As part of our pharmacological trials the pharmacokinetics of imipramine and desipramine have been investigated in detail, including the routine use of a "loading dose" (see #Z01 MH 01850-01).

3. In collaboration with Dr. Post of the Biological Psychiatry Branch, NIMH, the evaluation of psychotropic drugs in the treatment of acute mania and schizoaffective psychosis has continued. The focus has been on a comparison of pimozide with lithium and chlorpromazine. Pimozide can now be concluded to be an effective antimanic and antipsychotic agent which has a considerable effective dosage range. Some individuals show maintenance antipsychotic effects on 1-2 mg/day whereas treatment of acute mania may require 16-20 mg/day. The onset of the antimanic action of pimozide is significantly more rapid than that of lithium but at three weeks the overall antimanic effect of lithium is superior. Pimozide's antipsychotic potential is equivalent to chlorpromazine and its effects are most dramatic in "atypical" manias with schizoaffective features.

4. In collaboration with Dr. Donnelly of SEH, NIMH and Dr. Murphy of the Clinical Neuropharmacology Branch, NIMH, several studies of psychological functioning in depressed and manic patients are underway. These studies include the Halstead-Reitan battery, a test of associative capacity thought to be a sensitive indicator of organic dysfunction, the Wechsler

Adult Intelligence Scale, and the MMPI. Preliminary results suggest that depressed patients show significant deficits on the Halstead-Reitan; in the case of the bipolar patients this deficit does not entirely return to normal during the immediate in hospital recovery phase. Studies of IQ have indicated normal ranges for manic-depressive patients, but a striking change (averaging 20 points on the full scale) in the same patient going from depression into hypomania. MMPI studies continue to indicate significant differences between unipolar and bipolar patients in a variety of personality parameters. Our preliminary finding that clusters of individual MMPI items predict subsequent clinical response, to lithium and to imipramine, has been replicated in an independent sample.

5. In collaboration with Monte Buchsbaum of the Section on Cognitive and Perceptual Studies, BPB, we have continued and extended our studies on the cortical evoked potentials in manic-depressive patients. Attention has focused particularly on the use of differences in the auditory evoked response as a predictor variable in antidepressant drug response. The pretreatment amplitude-intensity slope is significantly greater (augmentation) among lithium responders compared to non-responders, while in the case of imipramine, the reverse is found. Although these responder vs. non-responder differences are in the direction predicted by the unipolar-bipolar difference in AER response. This biological marker identifies drug responders more accurately than does the clinical unipolar-bipolar distinction. Nonetheless, differences in AER among unipolar and bipolar depressed patients are now well established, and within each of these populations AER has been shown to be associated with age of onset and the presence of a family history of affective disorder. In addition, we have conducted longitudinal studies of AER in cycling manic-depressive patients and in normal controls and patients under stress. A collaborative study of pair responses in depressed patients has been completed; an inverse relationship between subjective "pain" complaints and sensitivity to experimentally induced pain has been found (for details of these studies, see project report from the Section on Cognitive and Perceptual Studies, Biological Psychiatry Branch).

6. In collaboration with Dr. Prange, the potentiation of tricyclic antidepressant response by co-administration of T_3 has been studied. In the 16 patients analyzed to date who were treated with T_3 after failing to respond to four weeks of tricyclic alone, 12 showed an antidepressant response within 7 days of T_3 administration.

In connection with all of the studies mentioned above our Branch focuses on a series of biochemical studies in patients described in detail elsewhere (indicated in parentheses):

a) Variations in plasma and urinary melatonin (Z01 MH 01851-01 CP)
b) Urinary excretion of MHPG as an index of norepinephrine turnover (combined central and peripheral) (Z01 MH 00448-05 CP)
c) Spinal fluid amines and amine metabolites (Z01 MH 00447-09 CP)
d) Multiple neuroendocrine measures, including responses to specific challenge tests, in plasma, urine and CSF (Z01 MH 00452-03 CP)

All biochemical parameters are compared across clinical state, diagnostic subgroup, variables of age, sex and family history, as well as drug versus non-drug conditions and drug response.

In addition to our attention to new biological techniques we continue to expand and improve our clinical descriptive capacities using more sophisticated diagnostic and rating instruments to provide reliable data for factor analysis and cluster analysis. It is expected that the application of these more advanced statistical techniques will go far in clarifying the questions about meaningful subgroups in affective disorders. Recently a protocol for short-term admissions (1 week, mostly on an outpatient basis) has been approved and a vigorous patient recruitment effort is underway under the direction of Dr. Cowdry. As part of this effort, a community collaboration group is being formed, comprised of local private and clinic psychiatrists. The increased number and variety of depressed patients will allow for a broader base to address the definition of subgroups in affective disorders using selected biochemical parameters.

Publications: Z01 MH 00446-09 CP

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00447-09 CP

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)
Cerebrospinal Fluid Amine Metabolites and Amines in Mental Illness

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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OTHER:	Rex Cowdry	Staff Psychiatrist	CP/NIMH
	Thomas Wehr	Chief, Clinical Research Unit	CP/NIMH
	Paul Marangos	Chief, Unit on Neurochemistry	CP/NIMH
	Philip Gold	Chief, Unit on Neuroendocrinology	CP/NIMH
	Robert Post	Chief, Clinical Research Unit	BP/NIMH
	Edna Gordon	Chief, Unit of Clinical Biochem.	LCS/NIMH
	Daniel van Kammen	Chief, Unit on Neuropsychopharm.	BP/NIMH
	Francis Major	Clinical Associate	CN/NIMH
	Ray Lake	Staff Psychiatrist	LCS/NIMH
	Pauline Lerner	Staff Scientist	BPB/NIMH
	Michael Ebert	Chief, Sec. on Exp. Therapeutics	LCS/NIMH
	J. Ballenger	Clinical Associate	BP/NIMH
	David Sternberg	Clinical Associate	BPB/NIMH

COOPERATING UNITS (if any)
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LAB/BRANCH
Clinical Psychobiology Branch

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.2	0.8	

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☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Amine metabolites in cerebrospinal fluid (CSF) are studied in patients to assess neurochemical alterations which may be associated with clinical diagnoses (particularly affective illness, schizophrenia, personality disorders, and alcoholism), biological subgroups, or drug treatment. Probenecid, which inhibits the removal of the metabolites from CSF into the blood, is administered as part of these studies. A study of CSF in normal volunteers is underway both as controls for psychiatric groups and as a separate population for study. In patients with personality disorder, 5-HIAA showed a strong negative correlation with a history of aggression. Low DBH was associated with elevated MMPI profiles and with the development of a psychosis during disulfiram treatment. Several studies explore the use of pretreatment CSF metabolite values in predicting drug response. Neuroendocrine studies suggest heightened serotonergic activity in some unipolar endogenously depressed patients. Physostigmine affects CSF, HVA and MHPG, suggesting a cholinergic influence on dopaminergic and noradrenergic systems. Simpler fluorometric procedures for 5HIAA and HVA were found to correlate highly with the mass spectrometry method, especially in the higher ranges achieved with probenecid.

The Chief, CPB, serves as the coordinator for CSF studies in the Division; the Unit on Neurochemistry, CPB provides sample processing and assays (the latter in collaboration with Edna Gordon of the LCS); this Branch also provides central data storage and analysis for CSF results. The major collaborating groups for the CSF studies are the Sections on Psychobiology (Dr. Post) and Neuropharmacology (Dr. van Kammen) of the Biological Psychiatry Branch and the Clinical Neuropharmacology Branch (Drs. Murphy and Major).

This project explores the neurochemistry of various psychiatric conditions using clinical methods previously developed in this Branch, and lab techniques developed in the Branch and in the Unit on Clinical Biochemistry, LCS.

The purpose of this project is the direct assessment of the functional state of brain neurotransmitter amines in patients by assay of levels of the amines and their major metabolites in the cerebrospinal fluid (CSF) under baseline conditions and following the administration of probenecid. The metabolites under study are 5-hydroxyindoleacetic acid--5HIAA, the major metabolite of serotonin, homovanillic acid--HVA, the major metabolite of dopamine, and 3-methoxy-4-hydroxyphenylglycol acid--MHPG, and 3-methoxy-4-hydroxyphenylacetic acid--VMA, the major and minor metabolites, respectively of norepinephrine; in addition, norepinephrine itself is measured in collaboration with Dr. Ray Lake of the LCS, and DBH is assayed in collaboration with Dr. Pauline Lerner of the BPB.

The probenecid technique itself has been a subject of more extensive investigation. Originally, probenecid in doses of 100 mg/kg p.o. was thought to provide essentially complete blockade of acid metabolites from CSF, thus providing a measure of the rate of production and metabolism of the parent amines. Our more recent work, in collaboration with Michael Ebert of the LCS, demonstrates a highly significant positive correlation between CSF levels of probenecid and levels of both 5HIAA and HVA in a group of drug-free depressed patients, accounting for between 10% and 25% of the observed total variance in metabolite levels after probenecid. This implies either that the blockade induced by probenecid is not complete in these patients, or that higher levels of probenecid induce increased acid metabolite production.

In contrast to the finding in depressed patients, no correlation between CSF probenecid levels and acid metabolite levels in schizophrenic patients was found, suggesting either possible membrane transport differences, or differences in central sensitivity to probenecid among diagnostic subgroups. Patients with primary affective disorder treated with lithium similarly show no correlation of CSF probenecid with acid metabolite levels, in contrast to the high correlation noted in the same patients in the drug-free state, suggesting that lithium may stabilize either the acid membrane transport system, or the CNS neurotransmitter response to administered probenecid. Finally, a variety of psychoactive drugs were noted to lower CSF probenecid levels significantly, a factor which must be taken into account in studies of amine metabolite changes associated with drug treatment.

Some of these issues regarding diagnostic differences will be more easily resolved if the probenecid technique is employed in normal volunteers. A preliminary study, in collaboration with Dr. Robert Gerner at UCLA, has provided some initial values from normal controls, but a larger group would be needed for meaningful comparisons with our psychiatrically ill populations. So far 30 normals have been studied at NIMH.

Partially as a result of discovery of confounding sources of variance in the probenecid methodology, we have placed increasing emphasis on baseline studies. The use of gas chromatographic/mass spectrometric techniques for assaying amine metabolites produces the requisite precision in measuring the low levels found in baseline lumbar punctures.

In collaboration with the other units as noted above, we have continued the exploration of CSF amine metabolites within and between various diagnostic groups (affective illness, schizophrenia, schizoaffective disorders, character disorders and alcoholism) with the emphasis on primary affective illness. In addition, in collaboration with Dr. James Ballenger, we have begun studying amine metabolites in the spinal fluid of normal volunteers, relating the baseline values to a variety of personality variables. Thirty volunteers have been studied to date. By broadening our study populations, we hope both to develop control group values for comparison with other diagnostic groups and to identify features of personality or behavior which may be related to monoaminergic function across diagnostic groups.

Some promising findings have appeared in this regard. In collaboration with the National Navy Medical Center, a study of individuals with personality disorders and/or alcoholism has been completed. In this population, the serotonin metabolite 5HIAA was found to be strongly negatively correlated with a life history of aggression, directed outward (violence, criminal acts) and/or inward (suicidal behavior). This finding is consistent with animal data regarding the inhibitory influence of serotonergic systems on aggressive behavior, and may have implications for the treatment of some forms of violence, particularly episodic. Also, in another study in this population, individuals with CSF dopamine beta-hydroxylase (DBH) levels below the group median were found to have a markedly elevated-MMPI profile, with particularly significant elevations on scales Pd, Pt, and Sc (compared to the subjects with DBH levels above the median). Since we have found mean DBH levels to be similar in different psychiatric diagnostic groups, DBH may be a marker for certain personality features of behaviors which occur independently of diagnosis. In alcoholics, low CSF DBH has also been found to be strongly associated with the development of psychosis during treatment with disulfiram, a DBH inhibitor. In contrast, individuals with high pretreatment DBH levels develop few adverse psychiatric effects. Thus, further inhibiting the already low levels of DBH activity may result in further increases in dopamine and decreases in noradrenaline. A variety of lines of reasoning suggest that the increased dopamine levels may be central to the development of psychosis, perhaps mediated through the formation of abnormal metabolites.

CSF norepinephrine (NE) has been studied by Dr. Robert Post in collaboration with Dr. Ray Lake. NE was higher in mania than depression. Depressed patients' NE levels were not significantly different than neurological controls. CSF NE has been found to correlate with CSF DBH in another study, done collaboratively with Dr. Lerner.

Neuroendocrine parameters in affective illness and their relationship with CSF amine metabolites have been studied by Dr. Philip Gold. He has found a negative correlation between CSF 5HIAA and the TSH response to TRH in unipolar patients; this association supports a postulated inhibitory serotonergic influence on TSH response, and suggests that the blunted TSH response in unipolar depressed patients as a group may reflect serotonergic hyperactivity. This would be consistent with preliminary results linking high urinary free cortisol with elevated 5HIAA in some unipolar endogenous depressed patients.

In collaboration with the BPB, CSF calcium was found to be related to the severity of the depression within a group of patients with primary affective disorder, but did not distinguish depressed patients from other diagnostic groups (Jimerson, et al.). Previously we had reported that hypocalcemia develops in plasma and CSF following 3 to 5 ECT treatments, coincident with the onset of therapeutic effects (Carmen, et al.). This finding may be related to amine neurotransmitter activity.

A collaborative study with Drs. Kenneth Davis and Leo Hollister at the Palo Alto VA Hospital demonstrated that probenecid-induced HVA and MHPG levels were higher in individuals given physostigmine then in those given saline (placebo). This suggests a cholinergic influence on both dopaminergic and noradrenergic functioning.

Drug response prediction using CSF metabolites continues to be a major area of interest. Antidepressant response to piribedil, a dopamine receptor agonist studied in collaboration with Dr. Robert Post shows a strong negative correlation with pre-treatment HVA. This finding suggests that low pre-treatment HVA reflects decreased central dopaminergic activity. The clinical improvement observed relates to the reversal of this decreased HVA, consistent with a negative feedback mechanism regulating the dopaminergic system.

Further research is underway to explore the usefulness of CSF 5HIAA levels in predicting responses to tricyclic antidepressants. Our results suggest that unipolar patients with low 5HIAA levels are more likely to respond to amitriptyline than those with high CSF 5HIAA, consistent with the observations that amitriptyline is a drug with relatively serotonergic effects. The opposite pattern seems to hold for imipramine, a relatively more noradrenergic drug. Attempts are in progress to combine this prediction data with predictions based on urinary norepinephrine metabolites, and to expand these metabolite studies to the prediction of lithium response. The introduction of a crossover study comparing desmethylimipramine (a

specific noradrenergic agent) with zimelidine (a strongly serotonergic agent) may help clarify these relationships.

Finally, several recently developed assays in CSF are foci of special interest - particularly a mass spectrometric assay for melatonin (see report Z01 MH 01851-01 CP) and assays for a variety of neuropeptides, such as vasopressin and somatostatin.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00448-05 CP

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)
Urinary Norepinephrine Metabolites in Affective Disorders and Normal Controls

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	Rex Cowdry	Staff Psychiatrist	CP/NIMH
	Philip Gold	Chief, Unit on Neuroendocrinology	CP/NIMH
	Thomas Wehr	Chief, Clinical Research Unit	CP/NIMH
	Athanasios Zis	Guest Worker	CP/NIMH
	Robert Post	Chief, Clinical Research Unit	BPB/NIMH
	H. Dekirmenjian	Research Biochemist	ISPI

COOPERATING UNITS (if any)
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Clinical Psychobiology Branch

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1.3	0.7	

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords) This ongoing project evaluates a wide variety of factors which contribute to the urinary excretion of 3-methoxy-4-hydroxyphenylglycol (MHPG), the norepinephrine metabolite thought best to reflect brain turnover. New findings or replications of earlier ones are as follows: (1) in bipolar patients, 24-hr urinary excretion is low in depression and high in mania but does not predict mood changes; (2) the altered circadian rhythm of MHPG in depressed patients is the basis for a collection of 11p.m. - 7a.m. urines which may prove especially helpful in current outpatient or normal volunteer research; (3) MHPG changes induced by physical activity can be shown in "well-state" outpatients with a history of manic-depressive episodes; (4) there is a modest positive (0.56) correlation between MHPG of depressed patients and nurses' ratings of their anxiety; (5) there is no correlation between urinary MHPG and urinary free cortisol which is presumably under serotonergic control; (6) systematic comparisons of quantitative methodology indicate that gas chromatography is a reliable means of measuring MHPG and that comparison with GC-MS provides a systematic factor of 0.83 to arrive at a correct absolute value.

Project Description: ZO1 MH 00448-05 CP

The purpose of this continuing project is an extensive study of norepinephrine metabolites (particularly 3-methy-4-hydroxyphenylglycol--MHPG) in patients and normal volunteers. MHPG is the norepinephrine metabolite thought best to reflect brain norepinephrine turnover--the most recent clinical studies by Ebert and Kopin in the LCS using radioisotopes to study turnover indicate that a significant proportion of MHPG appearing in the urine originates from brain pools of norepinephrine.

We have continued a detailed investigation of factors which influence MHPG excretion including activity , diet and anxiety. In order to use MHPG excretion as a tool in evaluating the hypothesis of altered norepinephrine function in depression and mania, the influence of such non-specific factors on MHPG excretion must be understood. State-dependent changes in MHPG excretion may be of even greater importance as suggested by our earlier longitudinal studies in patients with rapid cycles.

The methods for urinary MHPG studies in patients and normal volunteers have been described in the description for this project covering the period July 1, 1975 - June 30, 1976. In brief, daily urine samples are collected either in 3-hour segments or as 24-hour pools, measured, refrigerated with preservative, aliquoted, frozen and analyzed for MHPG. We are also investigating the possibility of 11 p.m.-7 a.m. urine collections. Six areas of study may be summarized:

(1) Longitudinal studies in manic-depressive patients with rapid cycles have been extended. In these patients at least one complete cycle with depression and mania has been investigated. Results confirm earlier trends such that (a) there is significantly more MHPG excreted in the urine during the manic phase than in the depressed phase; (b) MHPG alteration does not precede switches into mania or depression so that MHPG can not be used as a predictor of a switch; (c) using a new sample of depressed bipolar patients our earlier findings that bipolar depressives have significantly lower MHPG than unipolar depressives has been replicated.

(2) Routine application of the patient activity monitor (PAM), a non-telemetric computer-based motor activity monitor, has recently been possible. With this device, the clear rhythm in urinary MHPG already reported for normal volunteers has been shown to follow their regular pattern of activity. Although not originally demonstrable, as similar circadian rhythm in MHPG associated with the level of activity has been found in depressed patients. The finding of a difference in the timing of the MHPG circadian rhythm in volunteers vs. depressed patients has been replicated. We are now assessing the 11 p.m.-7 a.m. urine specimens which are more easily obtained in an outpatient setting than 24-hour urines. Depressed patients consistently show a lower urinary MHPG than our controls in collections taken at this time.

(3) Further investigation of effects of diets confirms our findings that depressed patients but not normal volunteers will have a nearly doubled urinary MHPG excretion when switched from a low to a high monoamine diet. Earlier we have shown that physical exercise increased MHPG in patients but

not in volunteers. In order to see if this responsivity of depressed patients' MHPG to exercise was only characteristic of the depressed state, former "recovered" patients have been reinvestigated (Dr. Zis, see also Z01 MH 00449-04). Three out of four "well-state" patients studied to date show increase urinary MHPG with exercise suggesting that altered MHPG responsitivity is not limited to periods of clinical depression. We have also found that on inpatients there is a 0.56 correlation between increases in MHPG during depression and nurses' ratings of anxiety.

(4) Tests of patients' abilities to "buffer" neurohumoral systems have been continued. Current findings show that urinary free cortisol does not correlate with urinary MHPG consistent with the animal data that cortisol production is not regulated by norepinephrine but by serotonin (Z01 MH 00452-03).

(5) Special attention has been given to quantitative methodology in light of occasional discrepancies between laboratories as to absolute values. Our earlier finding of a 1.0 correlation of MHPG determined by GC with an EC detecter and by GC-MS (Dr. Gordon) has been replicated as has the finding that the absolute value obtained by GC are 125% of that obtained by GC-MS. We have completed a collaborative study with Dr. Dekirmenjian's laboratory at ISPI in Chicago and find a good correlation between ISPI's GC and our GC results.

Goodwin, F.K. and Potter, W.Z.: The biology of affective illness: amine neurotransmitters and drug response. In Depression: Biology, Dynamics and Treatment. Cole, Frazier, and Schatzberg (Eds), Plenum Press, New York, 1977, pp. 41-73.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00450-04 CP

PERIOD COVERED

October 1, 1977 - September 30, 1978

TITLE OF PROJECT (80 characters or less)

Biological Rhythms in Affective Illness

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	Howard Hoffman	Statistician	BB/NICHD
	J. Christian Gillin	Chief, Unit on Sleep Studies	BPB/NIMH
	Elliot Weitzman	Chairman, Dept. of Neurology	A. Einstein Med. School

COOPERATING UNITS (if any)

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1.9	1.1	

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords) Basic research in biological rhythms can be related to affective illness with respect to (1) the inherent cyclicity of the illness (itself a type of biological rhythm) and (2) the involvement of disturbed circadian rhythms (24-hour cycles) in its pathophysiology. This project is designed to explore these connections by interrelating behavioral, physiological and biochemical changes observed (1) through the cycle of the illness and (2) through the course of the 24-hour day. Results to date indicate (1) that in a subgroup of female bipolar patients the natural rate or frequency of cycling between mania and depression is accelerated 4-fold by maintenance tricyclic antidepressants. The effects of tricyclics on the frequency of free-running circadian activity rhythms in hamsters is currently under study. (2) depressive and manic patients show state-dependent phase shifts (changes in timing) of circadian rhythms in temperature, motor activity, sleep architecture and neurotransmitter metabolites; in this circadian desynchronization the rhythm is not "locked in" to the environmental 24-hour day-night cycle, but tends to drift in and out of phase. (3) In phase shift experiments it has been possible to switch patients out of depression by directly manipulating the timing of circadian rhythms by changing patients' sleep schedules.

Project Description: Z01 MH 00450-04 CP

Epidemiological evidence has emphasized the essential cyclicity of both unipolar and bipolar affective illness. Since manic-depressive illness is a periodic illness, a study of the role of biological rhythms in its pathophysiology and pathogenesis is important. This project involves longitudinal studies of behavioral, physiological, and biochemical variables in inpatients and outpatients who have relatively frequent mood cycles. Focus is on both circadian (i.e., 24-hour) and longer rhythms, requiring both intensive data collection (multiple sampling within the same day) and longitudinal data collection over time (in several patients, we have obtained daily measures over many months).

Behavioral parameters under study involve both nurses and self-ratings, as well as motor activity monitoring using a nontelemetric solid state, computer-based device developed at NIH. Physiological parameters include EEG sleep studies, temperature, biochemical variables including urinary 3-methoxy-4-hydroxyphenylglycol (MHPG--the urinary metabolite thought best to reflect brain norepinephrine metabolism), melatonin and (less frequently) CSF amine metabolites. These data are subject to time series analysis in order to characterize circadian and longer rhythms.

Findings in these studies can be described under several headings:

1. We had previously identified a group of bipolar cycling patients who show circadian rhythm desynchronization in temperature and in motor activity and have now extended the findings to additional patients. In these patients, circadian rhythms are not locked into phase with the environmental day-night cycle, but tend to drift gradually in and out of phase. In this respect, these patients behave as if they had been cut off from all environmental light and time cues; these findings suggest some disconnectedness in the systems responsible for integrating environmental cues into the regulation of biological rhythms. The systems involved in this integration include hypothalamic clock mechanisms, the pineal gland and connecting central norepinephrine pathways.

2. We have now completed a study designed to compare diurnal patterns of urinary MHPG excretion using three-hour urine collection periods around the clock in depressed patients and normal controls. We found a diurnal pattern for urinary MHPG in normal controls with a maximum in the daytime and a minimum at night. Depressed patients as a group show an alteration in timing of diurnal MHPG variation (an earlier peak) as well as amount (lower mean). Compared to controls, depressed patients show decreases in MHPG which are maximal in the late evening. This finding is consistent with the hypothesis that diminished central noradrenergic activity is responsible for abnormal cortisol secretion and decreased REM sleep latency in the late evening reported to exist in depressed patients.

3. Continuous monitoring of motor activity has become a principal focus of ongoing longitudinal studies of affective disorder. Activity is measured with a small, ambulatory solid state device worn on the wrist. Data is periodically retrieved and analyzed by computer. Continuous monitoring each 15 minutes makes it possible to measure the amount and, equally important, the timing of activity in patients as clinical state changes.

The level of motor activity and the temporal distribution of activity, like other physiological functions, is regulated by neural mechanisms in the central nervous system with known input from noradrenergic, serotonergic, dopaminergic and cholinergic neurotransmitter systems. In particular, the 24-hour activity-rest cycle is driven by a hypothalamic "pacemaker" in animals and has been the focus of intensive investigation in basic biological rhythm research. Recurrent depressive and manic-depressive illness may be conceptualized as disorders of this activity-rest cycle "pacemaker" in as much as activity monitoring has revealed: a) consistent state-dependent changes in activity; b) changes in timing (phase shifts or frequency modulation) of the daily onset of activity; c) "splitting" of the daily activity phase into two components in stable hypomania; and d) state-dependent changes in the ratio of the length of the daily activity phase to the length of the daily rest phase in various patients studied longitudinally to date.

It is of particular importance that each of these findings can be induced experimentally in animals with environmental (light, schedules), chemical (endocrine, drug) and anatomical (hypothalamic lesions) manipulations. Thus, motor activity monitoring provides a direct bridge between disturbed behavior in psychiatric patients and experimentally-induced behavior in animals, and relates this behavior to central neuroregulatory systems which have been intensively investigated in animals for many years.

Using the existing NIMH activity monitor, we have extended our studies of cyclic affective disorders to several additional patients, some of whom show state-dependent circadian rhythm desynchronization. In order to further our understanding of mechanisms which regulate circadian activity rhythms, we have undertaken to study longitudinally (1) primary circadian rhythm disturbances in non-affectively ill patients such as insomniacs (one such patient who is unable to adhere to a 24-hour schedule and lives on a 25-hour "day" has been intensively studied for five months) (2) and normal humans who are free-running and paced entirely by their internal clocks in complete isolation from environmental time cues (in collaboration with Elliot Weitzman at the Laboratory of Human Chronophysiology, Albert Einstein Medical School, New York).

Monitoring of subjects for periods exceeding one year has made development of techniques of data reduction and analysis essential. Major progress in this area has been made during the past year. In collaboration with William Vaughn, computer programmer in the Technical Development Section, we have developed a series of interactive computer programs designed to display and analyze large amounts of activity data rapidly. For example, using graphic techniques ("actograms") which have become standard

means of representing longitudinal animal activity data, it is now possible to plot a year's continuous activity in a single diagram which coherently demonstrates trends in parameters of the activity rest cycle. Algorhythms which detect artifacts, determine length of sleep, times of awakenings, changes in levels of activity and distribution of activity through the day have also been developed. Changes in these variables are clinically relevant and it is expected that the instrumentation together with the automated data processing package may become useful and cost-effective in large scale clinical and investigational assessment of patients with regard to diagnosis, drug response and course of illness.

Limited data-storage capacity of the present monitor, which requires three times a week computer "read-out" has usually made outpatient monitoring impractical. A major development this year has been the design and imminent manufacture of 50 new monitoring units with extended electronic memory requiring only weekly"read-out". Large scale outpatient studies are anticipated in the coming year. Activity monitoring will prove to be the only continuous clinical data base for outpatient studies in the new ACRF.

In order to exploit the potential of motor activity monitoring as a behavioral bridge between psychiatric patients and experimental animals, we have constructed an animal laboratory designed, with Theodore Colburn and his associates at the Technical Development Section to monitor longitudinally the circadian activity-rest cycle in 16 hamsters in constant environmental conditions. The system monitors wheel-running activity continuously with a microprocessor which stores data on magnetic tape. Data can be analyzed and plotted using the same programs and format which have been developed for human studies and thus can be directly compared to human data. In this facility, which has just become operational, it will be possible to test the effects of chronically administered psychoactive drugs on the mechanisms which regulate circadian rhythms in these "free-running" animals. Lithium ion is already known to alter the intrinsic rhythm or frequency of the hypothalamic circadian pacemaker. We will be testing the hypothesis that tricyclic antidepressants, which alter the timing or phase of circadian rhythms in some affective patients as they respond clinically to the drug, and which induce rapid cycling in some patients, have fundamental effects on the intrinsic periodicity of the free-running hypothalamic clock in experimental animals.

A series of experiments designed to evaluate the effects of psychotropic drugs on hypothalamic clocks, and to examine the neuroregulation of hypothalamic clocks is planned.

4. Studies of the effects of weak entraining agents on free-running circadian rhythms in animals provide an experimental paradigm for the induction of long-range cyclic variation in levels of motor activity (the most clearcut "bipolar" feature of manic-depressive illness) complete with recurring "switch-over points". By progressive shifting of scheduled patient activities, it is possible to establish an analogous experimental condition

on an open ward. In four bipolar patients studied to date, it has been possible in this way to dissociate the activity rest cycle from the circadian temperature rhythm which then free-runs at period lengths greater than 24-hours. The four patients all showed mood changes from depression into mild to marked hypomania midway through the experimental rhythm desynchronization. Using a patient room which has been specially adapted for daytime sleep it has been possible to conduct a type of rhythm experiment heretofore only possible in isolation in underground caves.

The transient antidepressant effect of sleep deprivation therapy has been thought to be mediated by mechanisms which regulate the synchronization of circadian rhythms with the environment. We have recently tested this hypothesis with an experiment specifically designed to manipulate the timing or phase or circadian rhythms without decreasing sleep. Reasoning that sleep deprivation in man is equivalent to continuous light in animals, one would predict on the basis of animal experiments that continuous wakefulness induces a phase advance in circadian rhythms, along with increased overall activation (according to "Aschoff's Rule"). This hypothesis predicts that simply advancing a depressed patient's daily schedule several hours earlier than usual would, like sleep deprivation, cause a similar phase shift in circadian rhythms accompanied by increased activation. Sleep deprivation has only a transient (one day) antidepressant effect. In contrast to one night's sleep deprivation, however, the phase advance experiment can be sustained for many days and would be expected to have a sustained antidepressant effect. Recently a depressed patient who had consistently and dramatically responded to one night's sleep deprivation was placed on a six hour phase advanced daily schedule. She responded with a dramatic and sustained remission. Other depressed patients will be screened for similar phase shift experiments using the response to one night's total sleep deprivation as a predictor of response to circadian phase shifting. Future phase shift experiments will include EEG sleep monitoring; existing studies indicate that percentage REM and REM latency can be systematically altered by transmeridianal flight. Changes in these parameters would be expected to occur during phase shift experiments and would be of interest in view of numerous studies showing state-dependent changes in these variables associated with cyclic mood disorders.

5. One of our efforts to characterize circadian rhythm alterations in affective disorders uses integrated concentrations of blood constituents obtained through a recently developed ambulatory blood pump which continuously draws small amounts of blood from an indwelling catheter. Integrated sampling is a methodological advance which reduces the number of samples necessary to characterize circadian rhythms and reduces variability in the concentrations measured. The device is currently being used in studies of circadian neuroendocrine profiles of depressed and cycling patients and studies of melatonin levels in depressed patients. Six patients have been studied in both mania and depression to date, and samples will be assayed for GH, FSH, LG, TSH, PL and cortisol.

6. Our earlier observations that antidepressant medications alter the time course of recurrent affective disorders have now been extended to a larger group of patients. Tricyclics, have markedly accelerated mood cycle frequency from one cycle every few months to one cycle every few weeks or days in a subgroup of female manic-depressive patients. Therefore, these drugs appear to act by driving rather than counteracting the pathophysiological process underlying recurrent depressive illness. As mentioned previously, this process may involve changes in timing of circadian rhythms. This hypothesis will be explored in animal studies of the effects of antidepressants on the spontaneous (free-running) frequency of hypothalamic biological oscillators which drive the activity-rest cycle.

7. Working in Dr. Markey's lab, Dr. Lewy has developed a gas chromatographic mass spectroscopic (GC-MS) assay for melatonin (see melatonin project). This assay is highly sensitive and specific, well beyond any previously achieved assays for melatonin. Dr. Lewy has also developed a radioimmunoassay for melatonin and is using the GC-MS as a reference to evaluate the specificity of the immunoassay. Melatonin, produced by the pineal gland is thought to be an indicator, if not a mediator, of a central "biological clock". Many neuroendocrine substances appear to have either nyctohemeral or diurnal rhythms. Melatonin may regulate diurnal changes in levels of activity in the neurotransmitter and neuroendocrine systems which continue to be the focus of much of our clinical investigation. We intend to investigate this hypothesis in relation to manic-depressive mood cycles and in relation to other physiological (temperature, sleep stages, behavioral (activity), and neuroendocrine (prolactin, growth hormone, cortisol, and TSH) rhythms. Since the pineal is regulated by beta-adrenergic receptors, and since melatonin is a product of and a receptor-blocker of serotonin, we will have an output measure of adrenergic receptor sensitivity and will be determining levels of a possible endogenous regulator of serotonergic receptor sensitivity. These receptors may vary in their sensitivity with time of day.

Publications:

Wehr, T.A.: Phase and biorhythm studies of affective illness, pp. 321-324 in Bunney, W.E., Jr. (Moderator): The switch process in manic-depressive psychosis. Ann.Intern. Med., 87:319-335, 1977.

Wehr, T.A. and Goodwin, F.K.: Rapid cycling in manic-depressives induced by tricyclic antidepressants. Arch. Gen. Psychiat., 1978, in press.

Wehr, T.A. and Goodwin, F.K.: Biological rhythms and affective illness. In Weekly Psychiatry Update Series. Flach, F.F. (Ed.), Biomedia, Inc., New York, 1978, in press.

Wehr, T.A. and Goodwin, F.K.: Tricyclic antidepressants accelerate manic-depressive cycles. Chronobiologia, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 01850-01 CP

PERIOD COVERED

October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Clinical Pharmacology of Antidepressants

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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1.1	1.1	

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☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Although tricyclic antidepressants are very widely used in clinical practice the clinical pharmacology of these compounds is not well-understood. The purpose of the studies described in this report is to provide guidelines for the rational use of these drugs in the treatment of depression. Major foci during the past year include (1) pharmacokinetic prediction of steady-state concentrations; (2) assessing the role of plasma protein binding; (3) identifying active hydroxylated metabolites of tricyclic antidepressants; (4) describing the relationship between plasma concentration of a specific norepinephrine uptake inhibitor (desipramine) and biochemical effects and (5) establishing protocols for a clinical pharmacological evaluation of desipramine in normal controls.

PHS-6040
(Rev. 10-76)

Project Description: ZO1 MH 01850-01 CP

Research focusing on the clinical pharmacology of antidepressants has become possible following the development of a sensitive GC-MS assay in collaboration with Dr. Markey of the Laboratory of Clinical Science. The first clinical application of this assay was initiated by Dr. Muscettola (a former Visiting Scientist) in this laboratory in collaboration with Drs. Zavadil and Markey - a high correlation was demonstrated between plasma and CSF concentrations of imipramine and desipramine (see July '76 - Sept. '77 Report ZO1 MH 00446-08 CP).

Given the means of reliably measuring drug concentration, many studies have been undertaken. Rapid progress has been possible with the participation of Dr. Calil, a W.H.O. Fellow who joined the lab in October, 1977. The viability of the program has been recognized by designation of Dr. Goodwin as a preceptor for the NIGMS-sponsored clinical pharmacology program (PRAT program). An M.D. - Ph.D. Board Eligible psychiatrist matched with our Branch for a NIGMS-PRAT Fellowship, for 1979-81.

Two distinct approaches have been defined: I) Pharmacokinetic analyses designed to provide maximally effective utilization of proven antidepressants and II) comparisons of clinical and biochemical effects of specfic antidepressants in patients and normals as a means of studying the biochemistry of major affective disorders.

The major pharmacokinetic studies are as follows: (1) Following a model developed in Scandinavia for nortriptyline, Dr. Potter has shown that the area under the plasma concentration vs. time curve (AUC) following a single oral dose of imipramine in depressed patients accurately ($r = 0.9$) predicts ultimate steady-state concentrations. Twice weekly blood samples taken in the morning and used to measure steady-state concentrations show considerable variation. We have therefore drawn samples every two hours during a dosing interval to construct an interdose AUC which yields an accurate measure of steady-state. Recognizing that total AUC determinations require multiple plasma samples and may be of limited use for outpatients, an AUC based on three samples drawn at approximately 12 hour intervals beginning with 12 hours after a single dose has been shown to consistently predict 75% of the ultimate steady-state suggesting that a correction factor is possible.

All patients begin their tricyclic trial (we use desipramine, an active metabolite of imipramine) with a loading dose of drug followed by the determination of a 3-pt. AUC and the use of a 1/0.75 "correction factor" to select a drug dose designed to achieve a desired steady-state. In the three patients who have completed this prospective study, the loading dose prediction has proven to be accurate. This technique will be confirmed so as to assure from the outset that patients reliably achieve plasma concentration in the reported therapeutic range. In addition, we have shown that a single 24-hour measure of plasma imipramine or desipramine after a loading dose (100 mg p.o.) is a reasonably good predictor of ultimate steady-state. This provides a simple and potentially useful means of therapists selecting correct antidepressant dose at the outset of treatment. It is dependent primarily on the accuracy with which laboratories can measure the relatively low 24 hour plasma concentrations observed after a single dose.

(2) Since accuracy of determinations has such a potentially significant practical therapeutic value, we have initiated cross-validation studies of tricyclic determinations offered by two area commercial laboratories. These show that the reliability and precision of results from commercial laboratories are not yet good enough to use simple 24-hour levels to predict steady-state. Nor are single measures by these laboratories adequate to demonstrate that a patient is in the therapeutic range.

In a further effort to facilitate the process of making available accurate plasma measures, a cross-validation study with Dr. W. Jusko, Clinical Pharmacokinetics Lab, State University of New York, Buffalo, is underway. Dr. Jusko is using a HPLC method that, if effective, offers considerable long term advantages in terms of simplicity of equipment and level of necessary expertise.

(3) A related pharmacokinetic question of both theoretical and practical importance has concerned the role of plasma protein binding of tricyclic antidepressants. It has been asserted that by measuring total instead of "free" (non-protein bound) drug one may not get a meaningful measure of effective drug concentration. The binding project which was initiated last year has been completed. There were three important findings: first, careful attention to methodological issues allowed the demonstration that the percent of "free" drug did not show a wide range of interpatient variability as reported in an unreplicated study; second, the high affinity of imipramine for brain tissue in an in vitro competitive binding equilibrium dialysis (45X that for plasma) showed that plasma binding could not serve to limit the entry of drug into the CNS; third, using steady-state parameters an estimate of each patient's intrinsic metabolic capacity was obtained; as expected, this correlated inversely ($r = 0.98$) with imipramine concentration in the CSF. In other words, for a given dose, almost all variation in CSF drug concentration could be accounted for by individuals' variations in metabolism of the total drug. We have been able to conclude that measures of "free" drug are not indicated in studies relating concentration to clinical efficacy.

With respect to comparisons of clinical and biochemical effects of tricyclics, current studies are as follows: (1) A cross-over trial in which an experimental tricyclic specific for serotonin uptake inhibition, (Zimelidine) is compared to an established tricyclic antidepressant which is specific for norepinephrine uptake inhibition (desipramine). Since Zimelidine was only recently cleared by the FDA (May, 1978) trials have been restricted to desipramine. Preliminary results suggest that acute but not chronic desipramine may have some effect on certain neuroendocrine parameters (see Z01 MH 00452-02). The dose of desipramine used in these patients has been determined using the pharmacokinetic prediction described above.

(2) A protocol which describes the evaluation of acute and chronic effects of desipramine in normal volunteers has been approved. Multiple neuroendocrine effects of drug will be determined in conjunction with measurement of drug concentration. Thus, dose-response relationships in normals can be established. These will be compared to data from patients

(see above) presenting a means of assessing the possibility that affective illness produces an altered response to a drug which is known to effect norepinephrine turnover.

In collaboration with Dr. Judd (University of California at San Diego), the acute and chronic behavioral and biological effects of DMI in normal volunteers will be assessed; a large number of psychometric parameters are being employed. There are contradicting claims from poorly controlled studies concerning tricyclic antidepressants' effects on behavior, cognition and feeling in normals. Moreover, the time course of any action is in question which may be related to the apparent delay in onset of antidepressant effect. It would be very significant if certain alteration in normals only became apparent after 2-3 weeks. The collaboration described here should provide relevant data.

(3) The major theoretical question of the mode of action of tricyclic antidepressants has been addressed from both a conceptual and laboratory approach. As part of these studies, a critical review of the pharmacological and clinical studies of "new" antidepressants was completed by Drs. Zis and Goodwin. They found that both the claims of negligible amine effects and the claims of therapeutic equivalency to "standard" tricyclics were poorly supported by the data. (4) Moreover, in response to challenges to the clinical efficacy of tricyclics and the relationship to drug concentration, (most recently amitriptyline), we have critically evaluated the methodological pitfalls of such studies and concluded that spurious negative results may be expected.

(5) One such pitfall may be the tacit assumption that only certain forms of tricyclics are psychoactive. Using an *in vitro* synaptosomal system, we have shown that the presumably inactive hydroxylated metabolites of tricyclics show the same spectrum of amine reuptake inhibitory activity as do the parent compounds. Drug disposition studies in rats have shown that the hydroxylated compounds are present in appreciable concentrations in the central nervous system under steady-state conditions (the first such demonstration). And on a standard antidepressant screen (reversal of reserpine induced decreased activity and ptosis) the hydroxylated compounds appear to be active. The conclusions seem justified that hydroxylated tricyclics may possess antidepressant activity in man. The theoretical implications of this finding and the concentrations of these metabolites in man are being investigated. Preliminary results indicate that the hydroxylated metabolite may be present in concentrations equal to or greater than the desipramine itself at steady-state. Future clinical pharmacology experiments will be designed to assess whether these additional "active" forms of drugs correlate with specific effects *in vivo* since their physiochemical properties are different although distinct biological effects *in vitro* have not been demonstrated.

Publications:

Muscettola, G., Goodwin, F.K., Potter, W.Z., Claeys, M.M., Markey, S.P.: Imipramine and desimipramine in plasma and spinal fluid: relationship to clinical response and serotonin metabolism. Arch. Gen. Psychiat. 35:621-625, 1978.

Potter, W.Z. and Goodwin, F.K.: Antidepressant drug levels and clinical response. Lancet, i:1049-1050, 1978.

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Potter, W.Z., Muscettola, G., and Goodwin, F.K.: Binding of imipramine to plasma protein and to brain tissue: relationship to CSF tricyclic levels in man. Psychopharmacol., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 01851-01 CP

PERIOD COVERED

October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Cerebrospinal, Plasma and Urinary Melatonin in Affective Illness

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	Lawrence Tamarkin	Guest Worker	LDN/NICHD

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SUMMARY OF WORK (200 words or less - underline keywords)

The role of the pineal gland and its hormone, melatonin, has been difficult to define in humans, because the circulating levels of melatonin have been too low to measure. Recently, in collaboration with Dr. Sanford P. Markey of the LCS, we have developed a highly sensitive and specific gas-chromatographic-mass spectrometric (GC-MS) assay which requires less than 1 ml of human plasma, CSF, or urine. Since the development of this assay, melatonin research has been extended into two broad areas: (1) investigation into the physiologic role of melatonin (circadian secretion, the effects of light, drugs, sleep, the menstrual cycle, and the seasons of the year) and pathologic roles (precocious puberty, delayed onset of puberty, infertility syndromes, hypothalamic, pituitary, adrenal, and thyroid diseases, hypertension, breast and ovarian malignancies, as well as affective disorders and schizophrenia); and (2) investigations of this hormone as a "marker" for (a) the "biologic clock", (b) β-adrenergic receptor function.

PHS-6040
(Rev. 10-76)

Project Description:

Over the past 18 months, Dr. Lewy has developed and perfected a GC-MS assay for melatonin. Although radioimmunoassays (RIAs) and bioassays have been recruited for measurement of human plasma melatonin, problems with insufficient sensitivity, specificity, or efficiency have severely limited their use for routine analysis. Whereas GC-MS is theoretically the most specific of these techniques, attempts to apply it to melatonin analysis have also failed because of insufficient sensitivity. Using a new technique, negative chemical ionization, as well as improved chromatographic and extraction procedures, a GC-MS method has been developed which has the requisite sensitivity, specificity, and efficiency for the routine analysis of human plasma, CSF and urine. Less than 1 ml of sample is required, thus permitting wide flexibility of experimental design and repeated and frequent measurements. Since this is the most sensitive and specific of the current assays, low daytime concentrations can be accurately measured. Daytime values have been found to be a low as 1-2 pg/ml in contrast to nighttime values as high as 100 pg/ml. The range is consequently 20-to 50-fold. The RIAs and bioassays generally report higher values and a lower range, probably because of their lesser specificity. Our circadian curves reproduce pulsatile bursts during the night.

(1) A basic preliminary area of research has been started into the normal circadian secretion, and the effects of environmental lighting and sleep. To date, we have shown that humans differ from all other species, including primates, in that humans are closely tied to the biologic clock and not to environmental lighting. However, lighting and sleep appear to have some input into the control of melatonin secretion.

(2) In collaboration with Dr. W. Mendelson of the Sleep Lab (BPB), we have been studying the stages of sleep in relation to melatonin secretion. There appears to be a close correlation between REM sleep and pulsatile bursts of melatonin during the night. We will also be investigating the effects of melatonin infusions on the sleep of humans. Correlations with growth hormone and prolactin secretion are also in progress. Animal studies include the effect of melatonin on sleep in rats. To date there appears to be opposite effects of melatonin, depending on whether or not the species is nocturnal or diurnal.

(3) The response of melatonin secretion after an infusion of dopamine as well as in the other neuroendocrine stimulation tests is a major area of study (see report #Z01 MH 00452-03). Since the endocrine function of the pineal gland is as yet unknown, it is expected that these studies may increase our understanding of the interrelationships between the pineal and other glands. We are collaborating with other groups who have access to patients with various endocrinopathies, including hypothalamic-pituitary disease, thyroid disease, adrenal disease, precocious and delayed onset of puberty, and infertility.

(4) Other diseases of interest are hypertension and malignancy, particularly cancer of the breast and ovary. These are also being investigated in relation to melatonin secretion, in collaboration with the appropriate NIH institute.

Project Description: Z01 MH 01851-01 CP

(5) The effect of drugs, (particularly β-adrenergic agonists and antagonists) is being studied on patients with affective disorders and schizophrenia. In lower animals, melatonin synthesis and secretion is known to be controlled by a β-adrenergic receptor. Propranolol, a β-adrenergic antagonist and isoproterenol a β-adrenergic agonist, are currently in use in the treatment of various psychiatric syndromes. The effects of these drugs on melatonin levels is being explored. We are also studying the effect of psychotropic medication (tricyclic antidepressants, lithium, CPZ, MAO inhibitors), acutely and ch ronically. These medications have been thought to induce changes in β-adrenergic receptors.

(6) The melatonin assay gives us a powerful new tool in the in vivo evaluation of an important β-adrenergic receptor in patients. After infusing intravenous isoproterenol, melatonin secretion can then be measured and the amount of melatonin secreted is a reflection of the sensitivity of responsivity of the β-adrenergic receptor in the pineal. Since disease states and drugs may change the sensitivity of β-adrenergic receptors, we will be able to correlate receptor sensitivity changes directly with the disease states and the acute and chronic effects of medications. The neurotransmitter, norepinephrine, is the normal physiologic stimulant of the pineal gland, and therefore of melatonin secretion. Much attention has been focused on this neurotransmitter in conjunction with affective disorders and other psychiatric norepinephrine in vivo in man.

(7) Our manic-depressive patients provide a particularly appropriate population for the study of circadian rhythms and their disorders (see report #Z01 MH 00450-04). Using the acrophase as a marker for phase shifts, we have already found changes consistent with other phase shifts in temperature, activity (norepinephrine). Manic patients secrete twice as much melatonin as normals or depressed patients. We will be continuing these studies, not only with plasma measurement of melatonin, but also with urine measurements which offers the additional advantage of integrating the daily output of melatonin secretion.

(8) Studies of genetically hypertensive rats are underway in collaboration with Dr. Irwin Kopin. Melatonin measurement, before and after isoproterenol will be correlated with the degree of hypertension. Dr. Anna Wirz-Justice and Thomas Wehr are conducting studies on activity and the effects of environmental lighting and drugs on hamsters (see report #Z01 MH 00450-04). Melatonin will be measured as a biochemical marker for phase shifts in these animals, as well as a marker for β-adrenergic activity.

(9) In collaboration with Drs. Klein, Reppert and Tamarkin of the NICHD we are using the GC-MS assay to validate a RIA for melatonin, making appropriate adjustments in extraction techniques in order to insure specificity for a variety of tissues and species. We will recruit this assay to handle large volumes of samples when appropriate.

Publications:

Lewy, A., and Markey S.: Measurement of human plasma melatonin using negative ionization gas chromatography-mass spectrometry. Science, (in press).

Lewy, A.J., Wehr, T., Gold, P., and Goodwin, F.K.: Plasma and CSF melatonin in manic-depressive illness. Proceedings of the 4th International Cathecholamine Symposium, Asilomar, California, 1978, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00449-04 CP

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)
Outpatient Followup Studies of Manic-Depressive Patients and Families

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	Elliot Gershon	Chief, Unit on Psychogenetics	BP/NIMH
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	Eleanor Dibble	Social Worker	BP/NIMH
	Morris Parloff	Chief, Psychotherapy and Behavioral Intervention Center	CR/NIMH
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	Paul Grof	Professor of Psychiatry	McMaster U.
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COOPERATING UNITS (if any)
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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.3	1.3	

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords) The outpatient facility is a treatment and research resource for a variety of studies related to manic-depressive illness such as: (a) biological studies of the "well-state" which appear to be characterized by persisting abnormal responsivity to induced motor activity of norepinephrine turnover as measured by urinary MHPG alterations; (b) development of rigorous criteria for prophylactic lithium maintenance; (c) a collaborative retrospective analysis of data on 334 patients collected by Dr. Paul Grof which reveals that sex, polarity of first episode and age at onset are important clinical predictors of future clinical course; (d) continuing couples group therapy to better understand the usefulness of this treatment, as an adjunct to lithium maintenance; (e) investigation of the role of families' attitudes and perceptions of manic-depressive illness; and (f) prospective group therapy with bipolar couples who are expectant parents - to date, two out of three female index patients withdrawn from lithium experienced postpartum manic episodes. Other ongoing projects involve the study of long-term outcome as related to drug regimen, family dynamics and phenomenology of multigenerational families with affective illness.

PHS-6040
(Rev. 10-76)

During the past year, reorganization of the outpatient clinic occurred with the appointments of a Research Administrator (Dr. Zis) and a Clinical Administrator (Mrs. Davenport). Other personnel assigned to the Clinic part-time, are a psychiatrist and a nurse clinician. Currently, the clinic population if 65-70 outpatients who are seen in four montly medication supervision groups, three weekly psychotherapy groups, and one biweekly expectant parents group. Crisis intervention is also available. Because of limited staff time, new admissions are now being curtailed.

A major focus in the Clinic this past year has been on perfecting systems for data collection. A self-rating form was devised for outpatient use, incorporating features of the rating scale used on the ward for inpatients. An assessment form to note outpatient suitability for inclusion in research studies was also devised and is in use. Since some of the earlier patients had not received an RDC diagnosis during their inpatient hospitalization, it has been necessary to secure RDC data for these patients. Diagnostic and treatment evaluations by the consultants to the clinic were instituted this past year and are ongoing with material sent to the patients medical chart and available for staff discussion of clinical issues. Primarily through the initiative of the nurse clinician, an arrangement was made this year for use of the Clinical Center Phlebotomy team to draw bloods. In addition to serum lithium levels, we are also providing a new patient service by now securing monthly thyroid and creatinine levels. New patients will be randomly assigned to couple and singles psychotherapy groups in order to collect longitudinal behavioral data.

The availability of long-term follow-up data for large numbers of patients with affective disorders is of critical importance to our understanding of the basic illness process and therefore, to the interpretation of the increasing numbers of biochemical genetic and pharmacotherapeutic findings. For the clinician the choice of whether or not to place a given patient on prophylactic lithium maintenance involves at least an implicit estimate of what the natural course of the illness would be in the absence of the drug. Knowledge of the natural course of the illness is essential if he is to make appropriate decisions concerning long-term prophylaxis. The development of rigorous selection criteria for prophylactic lithium maintenance, deserves renewed focus in light of the recent reports regarding the effect of long-term prophylactic lithium maintenance on the kidney.

In an attempt to develop predictors of the subsequent course of the illness from characteristics of the first two episodes, we have been studying data collected on a large number of patient (229 unipolar and 105 bipolar) in three different countries (Czechoslovokia, Germany and Canada) by Paul Grof. Bipolar patients had a significantly lower age of onset than unipolar patients. No significant sex differences were detected in age of onset.

Although there was no difference between the average number of episodes experienced by male or female bipolar patients, males experienced mania more often, whereas depressive episodes were more common amongst females.

This was true for the polarity of the first as well as the polarity of subsequent episodes. In addition, polarity at onset correlated with polarity of subsequent episodes. Thus, patients with onset mania subsequently had significantly more manic episodes than those whose onset was a depression, and vice versa.

We also determined the probability of a relapse within 24 months based on the age of onset of the illness. There was a dramatic increase in the likelihood of a relapse with increasing age at onset. In addition, we found that age at onset predicted the number of episodes experienced in the first 5 years of the illness for bipolar patients. In addition to the effect of age of relapse frequency these statistical techniques are revealing that the duration of free interval can be partially predicted by the duration of the previous free interval.

The bipolar/unipolar dichotomy is based on the presence or absence of mania during the course of the illness. However, the first manic episode may not occur until late in the course of the illness as already pointed out by Perris. In our sample 15% of all bipolar patients did not switch into mania until after the third episode of the illness (false unipolar). Early discrimination between unipolar and bipolar diagnosis for patients is of critical importance for treatment as well as for interpretation of research findings. We are currently attempting to identify characteristics of the first two episodes in our false unipolars which differentiate them from the true unipolars.

The effects of motor activity on MHPG excretion has been studied in four patients who had been in a "recovery" phase for at least one year. Patients were on lithium and/or imipramine. MHPG excretion was considerably higher in 3 of these patients during the activity period, consistent with our finding in bipolar patients when actively depressed. This suggests that the differential MHPG response to experimentally induced increases in activity in patients with affective illness (i.e., not observed in normals) may be independent of the clinical state.

Psycho-social studies utilizing the clinic population are ongoing. An additional couples psychotherapy group was formed this year with a protocol designed to define a population of index subjects whose symptoms include "dysphoric mania". The couples groups are a major and rich resource for the study of the relationship of the illness in individual family members and family pathology. Interest continues in longitudinal studies of manic-depressive illness in multigenerational families. A manuscript - on the psychodynamic features of multigenerational families was submitted for publication in May 1978.

A collaborative study with the Psychogenetics Unit of the BPB is underway; in this study attitudes and perceptions of manic-depressive illness in families are examined, using patients in our couples group.

A second group, newly formed in the clinic this year as part of a joint collaborative service with the Psychogenetics Unit, consisted of four bipolar couples who were expectant parents. Three of the female index patients were withdrawn from lithium carbonate maintenance in the clinic when they indicated a wish to become pregnant. In the 4th month of the pregnancies, the clinic sponsored a childbirth education group, inviting the service of an RN childbirth educator from Parent Child, Inc. Evening sessions were held in which patients and their spouses participated. The couples completed a course in prepared childbirth, and discussed the psychology of pregnancy, the syndrome of post-partum "blues", and the high risk for the occurrence of post-partum affective disorder in this group of patients. Although it was our original hope to maintain the highly vulnerable patients off lithium, through lactation two of them developed post-partum manic episodes resulting in the hospitalization of one. A manuscript has been submitted for publication describing this experience, the variance in psychodynamic features and previous treatment history of the three patients, and offering recommendations for the care and treatment of bipolar patients considering parenthood.

We are also in the process of collecting data and reviewing medical charts to test the hypothesis that in a population of male manic patients, episodes of post-partum psychoses occur in proximity to births of children. This interest developed as a consequence of the observation of this phenomenon.

Study of the impact of adjunctive couples group psychotherapy on the prophylactic efficacy of lithium in bipolar patients continues. To assist us in these efforts we have established a collaborative relationship with Morris Parloff and his staff in the (Extramural) Clinical Research Branch so that our assessment techniques can be comparable to those to be used in the extramural collaborative study of drug-psychotherapy interactions.

Publications: ZO1 MH 00449-04 CP

Carlson, G.A., Davenport, Y.B., and Jamison, K.: A comparison of outcome in adolescent-and late-onset bipolar manic-depressive illness. Am. J. Psychiat. 134:8, 1977.

Zis, A.P., Grof, P., Goodwin, F.K.: The natural course of affective disorders: implications for lithium prophylaxis. Proceedings of the International Lithium Conference: Excerpta Medica (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00452-03 CP

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Neuroendocrine Studies of Major Psychiatric Disorders

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	Seymour Reichlin	Chief, Division of Endocrin.	Tufts U.
	Detlev Ganten	Professor, Dept. of Pharm.	U. of Heidelberg
	Gregory Brown	Chief, Dept. of Neuroscience	McMaster U.
	Gary Robertson	Professor of Medicine	U. of Indiana
	Samuel Yen	Chief, Dept. of Reproductive Endocrinology	UCSD

COOPERATING UNITS (if any)
Biological Psychiatry Branch, NIMH; Clinical Neuropharmacology Branch, NIMH; Tufts University; University of Heidelberg; University of California, San Diego; McMaster University, Ontario.

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.7	1.4	

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords) Hormones produced by the hypothalamic-pituitary axis (HP) are released by neurones which are modulated by monoaminergic neurotransmitters; moreover, these hormones can directly affect central biogenic amine function, producing distinct behavioral effects. This crucial interface between central nervous system function and neuroendocrine secretory systems is providing a basis for new methods in the study of psychiatric illness. Current studies concentrate on dynamic tests of HP function, measurement and administration of behaviorally active peptides, elucidation of the temporal organization of neuroendocrine function, and the relationship of neuroendocrine function to sleep and its disorders. Major findings this year are as follows: dopamine infusion significantly elevates plasma and platelet MAO; lithium blunts prolactin rebound following dopamine infusion suggesting a modulating effect on dopaminergic function in man; prolactin and gonadotropin response to dopamine in patients on neuroleptics resembles responses seen in idiopathic hyperprolactinemia; CSF somatostatin is higher in mania than in depression; growth hormone administration diminishes stage IV sleep; amenorrheic depressed women show neuroendocrine responses compatible with increased functional serotonergic activity.

Several aspects of the symptom complex of the functional psychoses, particularly affective illness, (such as disturbances in sleep, altered energy levels, changes in appetite and libido, diurnal variation in symptoms and amenorrhea) suggest hypothalamic dysfunction. Interest in the hypothalamic-pituitary axis (HP) has been further stimulated by recent findings that the monoamine neurotransmitters modulate the synthesis and release of a number of hypothalamic peptides and pituitary hormones. Thus, examination of pituitary hormones in plasma can shed light on the functional activity of biogenic amine systems. Moreover, these peptides and hormones can directly affect the brain neurotransmitter systems.

Several neuroendocrine strategies are currently in use; (1) dopamine infusions and administration of various releasing factors to test responses of the HP; (2) psychoactive drug effects on HP functions; (3) direct measurement in CSF or plasma of behaviorally active CNS peptides (4) assessment of the temporal organization of neuroendocrine functions; and (5) assessment of the relationship between neuroendocrine function and sleep.

Because of limited laboratory support personnel and the large volume of assays required to complete this neuroendocrine project, the Unit on Neuroendocrinology maintains collaborative and contractural arrangements with several university research facilities for neuroendocrine assays. We are fortunate to collaborate with scientists of outstanding caliber, including Dr. Reichlin, Tufts University, Dr. Yen, University of California, Dr. Brown, McMaster University and Gary L. Robertson, University of Indiana.

1) Dynamic tests of monoaminergically regulated HP systems:

a) A major emphasis this year is the evaluation of responses to a three hour infusion of dopamine to patients with affective illness and schizophrenia, and to control subjects. In normals, dopamine has been shown to inhibit prolactin release, presumably at the level of the pituitary, and to inhibit LH and FSH release, presumably at the level of the hypophysiotropic secreting cells.

Peak dopamine levels are achieved after one and one-half hours after which they fall substantially, despite continued dopamine infusion. To determine a possible mechanism for the fall in dopamine levels, platelet MAO was measured before, during and after dopamine infusion; significant elevation in platelet MAO was noted by 90 minutes in a group of control subjects. Further *in vivo* and *in vitro* studies are in progress to evaluate the mechanism of the MAO increment.

In collaboration with the BPB, schizophrenic patients on the neuroleptic pimozide were found to respond to dopamine infusion with an exaggerated prolactin response (decrease to the normal range) accompanied by paradoxical gonadotropin elevations. The magnitude of the acute prolactin

decrement and the rebound immediately following cessation of the infusion suggest increased dopaminergic receptor sensitivity during pimozide withdrawal. On the other hand, chronic lithium treatment significantly attenuates the prolactin rebound in the post-infusion period.

b) We have continued to explore the use of an "integrated" neuroendocrine procedure; this procedure utilizes arginine, a central releaser of growth hormone and prolactin, the hypothalamic peptide TRH, a releaser of prolactin and thyrotrophin stimulating hormone and LH-RH. The purpose of the timed infusion of these three trophic substances is to provide an evaluation of overall HP function. Data for a large number of patients in various clinical states and treated with various medications are now being analyzed Urinary excretion of the adrenal steroid, dihydroepiandrosterone sulphate has been found to be decreased in bipolar depressed women compared to controls.

c) We are studying neuroendocrine effects of apomorphine in affective illness, schizophrenia and normal subjects. Apomorphine, a dopamine receptor agonist has been shown to have significant effects on pituitary prolactin and growth hormone release. In collaboration with R. Post and D. Van Kammen of the BPB we are studying the pituitary response to apomorphine in various diagnostic subgroups and normals as a further dynamic test of dopaminergic functional activity in psychiatric patients. Full data analysis is awaiting completion of all the assays in Dr. Yen's laboratory.

d) Tests of the hypothalamic-pituitary-adrenal axis in affective illness have focused on urinary free cortisol (UFC) with and without dexamethasone suppression. Unipolar patients with psychosis have been found to have a very high UFC compared to unipolar patients without psychosis, or to bipolar depressed patients. Bipolar depressed patients show a significantly higher UFC than bipolar patients in the manic phase. Among unipolar patients, UFC shows a significant positive correlation with CSF 5HIAA after probenecid. Chronic lithium treatment reduced urinary free cortisol production, consistent with our CSF data indicating a decrease in serotonin turnover under these conditions.

2) Assessment of psychoactive drug effects on HP functions: The effects of commonly used psychoactive agents, such as lithium, imipramine, and MAO inhibitors, as well as of experimental drugs such as carbamezapine, piribedil, and pimozide are being assessed on each of our neuroendocrine challenge paradigms, including dopamine infusion, the "integrated" infusion (arginine, TRH and LH-RH) and apomorphine stimulation. Recently, it has been reported that desmethylimipramine, a specific noradrenergic uptake inhibitor, has acute effects on growth hormone release in normal subjects. Thus, studies of the neuroendocrine effects of an acute dose of DMI in patients and control subjects are currently underway.

3) Studies of behaviorally active CNS peptides.

a) The role of the CNS peptide vasopressin (VP) is under intense study, particularly in patients with affective illness. In a major review

we formulated a hypothesis suggesting a role for vasopressin in the regulation of affect in man. This published review stressed the functional relationship between vasopressin and many aspects of the symptom complex of affective illness, including alterations in the consolidation of memory, the modulation of the pain sensitivity, the synchronization of biological rhythms, the timing and quantity of REM sleep, and the regulation of fluid and electrolyte balance.

Several strategies are being employed to evaluate VP function in patients and control subjects. All patients receive hypertonic saline infusions to assess the centrally mediated "set point" for VP response to controlled increases in plasma osmolality. The effects of lithium, carbamezapine, and other psychoactive agents on this response is being studied. Because of the intimate relationship between VP serotonin and endorphinergic function, we are evaluating the effects of the narcotic antagonist naloxone on the VP response to hypertonic saline.

The CSF appears to be a major communication pathway for conveying VP to various areas of the brain where it may modulate cell aggregates controlling one or more physiological processes. Fortunately, vasopressin has been found in measureable quantities in human CSF and a narrow normal range has been established. In collaboration with Gary Robertson, we are measuring CSF vasopressin in all our patients and assessing the effects of clinical states and a variety of pharmacological agents on these levels.

b) CSF somatostatin levels are being studied in collaboration with S. Reichlin. Our interest in CSF somatostatin stems particularly from our earlier finding that the TSH response to TRH is significantly blunted in unipolar depressed patients compared to control subjects, and that the TSH response in manic patients tends to be lower compared to bipolar patients in the depressed phase. In a preliminary study, we find CSF somatostatin to be higher in manic patients than in bipolar depressed patients.

c) Neuroendocrine effects of opiate agonists and antagonists. Increasing evidence in experimental animals suggests that the endorphinergic system plays an important role in the modulation of a variety of neuroendocrine hormones, including vasopressin, prolactin, growth hormone, gonadotropins, and cortisol. In man, narcotic addicts are well known to produce several pronounced abnormalities of neuroendocrine function. Accordingly, we have begun studying the effects of naloxone on VP response to hypertonic saline, on baseline neuroendocrine function, and on TRH and LH-RH stimulated neuroendocrine responses. These data are currently being analyzed. Studies of the neuroendocrine effects of a potent enkephalin analog, FK 33 824 are just getting underway as are neuroendocrine studies of β-endorphin administration, done in collaboration with the BPB.

4) Assessment of temporal organization of neuroendocrine function. Neuroendocrine systems possess a characteristic temporal organization (daily, monthly and even yearly cycles). Studies are underway concentrating on describing the circadian organization of neuroendocrine function in mania

depression and following recovery and comparing these patterns to normal subjects. Studies of the neuroendocrine effects of sleep deprivation and continuous sampling over several days in rapidly cycling patients are underway.

5) Relationship between neuroendocrine function and sleep. Neuroendocrine function is characterized by distinct diurnal and nyctohemeral variations; the nocturnal variations of some hormones, notably growth hormone are related to the specific stage of sleep. Conversely, the timing of certain sleep stages may be related to the release of hypothalamic or pituitary hormones. In collaboration with C. Gillen we have examined the effects of growth hormone and LH-RH on sleep stages in normal subjects and have determined that (1) growth hormone administration significantly reduces stage IV sleep and (2) that LH-RH, produces a tendency towards reduced stage IV sleep in women.

6) Evaluation of neuroendocrine dysfunction in patients with affective illness. 'Psychogenic' amenorrhea is a frequent phenomenon that has been poorly understood from a neuroendocrine point of view. This profound disturbance in cyclic neuroendocrine function occurs with considerable frequency in patients with affective illness, particularly during the depressed phase. We have noted that depressed amenorrheic women show significantly higher CSF 5HIAA than depressed women with regular menstrual cycles. We have also shown that depressed amenorrheic women show a blunted TSH response to TRH negatively correlated with CSF 5HIAA, and an elevated urinary free cortisol production positively correlated with CSF 5HIAA. Taken together, these data suggest that amenorrheic depressed women have increased functional serotonergic activity, which is compatible with evidence derived from experimental animals that serotonin plays an essentially inhibitory role on gonadotropin release.

We have noted the occasional appearance of 'paradoxical' hyperthyroidism in patients on lithium in the outpatient department. The hyperthyroidism may be subclinical, detected only by thyroid function studies. Of interest is the fact that these episodes of subtle hyperthyroidism are usually accompanied by a manic episode. The mechanism of this hyperthyroidism and the associated breakthrough of mania are under study.

Gold, P.W., Goodwin, F.K., Wehr, T. and Rebar, R.: Pituitary-thyrotropin response to thyrotropin-releasing hormone in affective illness: relationship to spinal fluid amine metabolites. Am. J. Psychiat.134:1028-1031, 1977.

Gold, P.W., Reus, V.I., and Goodwin, F.K.: Vasopressin in affective illness - hypothesis. Lancet i:1233-1235, 1978.

Gold, P.W., Zis, A.p., Ballenger, J. and Goodwin, F.K.: Vasopressin in affective illness. Proceedings of the 4th International Catecholamine Symposium, Asilomar, California, 1978 (in press).

Mendelson, W., Gold, P.W., Slater, S.A., Gillin, C. and Goodwin, F.K.: Effect of growth hormone administration on sleep and memory in human subjects. Sleep (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 01831-02 CP

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)
Neuronal and Glial Isoenzymes of Enolase

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	Milton Brightman	Head, Section on Neurocytology	LNNS/NINCDS
	J. Barker	Medical Officer	LNP/NINCDS
	C. Schengrund	Associate Prof. Biochemistry	Hershey Medical School
	A. Parfitt	Research Investigator	AFRI
	G. Giotti	Research Biochemist	Salk Institute

COOPERATING UNITS (if any)
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0.9	0.5	

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This study involves determining the physiological role of two proteins isolated from brain, a neuron specific enolase (NSE) and non-neuronal enolase (NNE). The former is a specific neuronal antigen and the latter a glial antigen. Studies are aimed at elucidating the physiological significance of these strictly segregated cellular isoenzymes as well as their utilization as molecular markers in probing the functional organization of brain. Developmental, immunocytochemical and functional studies have established that NNE and NSE represent the first example in neurobiology of strict glial and neuronal markers respectively. NSE is only present in highly differentiated neurons. NSE has also very recently been shown to be present in APUD (amine precursor reuptake and decarboxylation) cells, a finding that supports the contention that neurons and neuroendocrine cells are closely related.

PHS-6040
(Rev. 10-76)

Project Description: Z01 MH 01831-02 CP

The study of brain function at the molecular level presents probably the most difficult problem in the realm of biological research. The myriad of cellular types, morphological complexity and elaborate interconnections coupled with the almost infinite diversity and complexity of functions ascribed to the brain make it an enormously elusive organ to study. In order to extract useful and valid information relating to brain function, it is necessary that one ask specific questions and attempt to isolate the system and parameters under study.

An approach that has been shown to be valuable in studying brain function at the molecular level is the functional characterization of proteins that are only found in brain. The assumption is that such proteins will subserve functions specific to brain, and that the functional characterization of these molecules will lead to a better understanding of brain function.

Work begun by Dr. Marangos at the Roche Institute and continued here in our laboratory has led to the isolation and characterization of a brain specific protein. This protein is a dimer of two 40,000 M.W. subunits. A specific immunoassay has been developed for the protein which made it possible for us to demonstrate that it is specific to nervous tissue. Immunocytochemical analysis has further revealed that the protein is only present in neurons, and accordingly, it has been designated neuron specific protein or NSP. Further work has revealed that it is a neuron specific form of the glycolytic enzyme enolase. Accordingly, the protein is now designated neuron specific enolase or NSE.

Brain tissue, particularly the neuron, is among the most metabolically active tissue in the body. More than 20% of total tissue oxygen comsumption is accounted for by the brain although it represents only about 2% of the total body weight. Glycolysis is a very important pathway in brain metabolism since the only substrate of energy metabolism known to traverse the blood-brain barrier is glucose. Any alteration of glycolytic flux would therefore effect oxidative metabolism (TCA cycle) as well as causing an alteration in the entire energy generating (ATP generating) process. The regulation of glycolysis in the neuron therefore becomes critical since an enormous amount of ATP is required for such diverse neuronal life processes as maintenance of membrane polarity, transmitter synthesis, release, reuptake, etc.

With the discovery that NSE is a neuron specific form of enolase it became important to determine if other forms of enolase exist in brain. Since NSE is strictly neuronal it follows that glial cells should have another form of the enzyme. We have determined that three forms of enolase exist in rat brain, one of which is NSE. We have purified a second enzyme species and raised specific antibodies against it. Immunocytochemical studies utilizing the peroxidase-anti-peroxidase staining technique have recently shown that this other enolase form is only found in glial cells. It is designated non-neuronal enolase or NNE.

The structural analysis of NSE and NNE has been completed with the results establishing that each protein is the product of a separate gene. NSE is a dimer composed of two γ subunits ($\gamma\gamma$), and NNE is a dimer composed of two α subunits ($\alpha\alpha$). The third brain enolase has been shown to be a hybrid designed $\alpha\gamma$. These results coupled with the anatomical studies establish that the γ subunit is only synthesized in neurons and the α subunit in glia. NNE and NSE therefore represent the first functional neuroanatomical markers for glial and neuronal cells respectively.

Collaborative studies are in progress with Donald Schmechel of the LCS (NIMH) and Milton Brightman of NINCDS using NNE and NSE to study specific functional morphological and developmental questions in rat and monkey brain. The studies include the appearance of NNE and NSE during development, alterationsin NNE and NSE levels (determined by RIA and staining) in specific brain areas in response to specific physiological manipulation; (i.e., blinding - optic lobes, olfactory stimulation - olfactory bulbs and chemical lesioning, kainic acid, 6-OH dopamine, etc.). Preliminary results indicate that NSE levels are affected in these procedures.

Very interesting results have been obtained in studying the developmental profiles of NNE and NSE in rat and monkey brain. This study is in its final stages and it has been shown that NSE is only present in the fully differentiated, functional neuron. The process of neuronal maturation in both rat and monkey brain has been shown to involve a switch from NNE to NSE. The immature neuron makes NNE and during development and differentiation looses its ability to synthesize NNE and starts producing NSE. These results are consistent with the cell culture experiments described in last year's report where it was shown that undifferentiated neuroblastoma cells possess NNE and differentiation leads to increased NSE.

The studies thus far described indicate that NSE is specifically required by the functional neuron. An interesting question is what distinguishes NSE from NNE that suits this form of enolase to the neuronal physiology? Our efforts in this area have established that NSE is distinct kinetically from other enolases in that it has a higher affinity for its cofactor Mg^{++}. More importantly, recent studies have shown that NNE and NSE differ greatly in their respective stability toward chloride salts. NNE is rapidly inactivated in the presence of 0.2M KCl, whereas NSE is unaffected. In light of the high chloride concentration known to exist during neuronal stimulation it appears that the unique stability of NSE to chloride suits it to the neuronal environment. NSE has also been shown to be much more resistant to temperature inactivation than NNE.

Our ability to measure small amounts of NNE and NSE using RIA has been greatly enhanced by the development of a new double antibody solid phase RIA. This assay has been developed during the last year and enables us to measure NNE and NSE in rat, monkey and human brain. The use of this improved assay coupled with the immunocytochemical staining methodology has led to the demonstration that NSE is present in certain specialized

neuroendocrine cells, namely the APUD (amine-precursor-uptake and decarboxylation) cells. The areas that have been documented by both RIA and staining to contain NSE include adrenal medulla (chromaffin cells), pineal (pinealocytes), thyroid (c-cells), pituitary (β-lipotrophin and ACTH cells) and the pancreas (Islet of Langerhans). This very interesting group of cells has been implicated as being "neuron-like" based on their neuroectodermal origin, ability to metabolize amines and their secretion of neuropeptides. The fact that the APUD cells contain a neuron specific antigen is a direct validation of the APUD concept. The presence of NSE in these "neuron-like" cells indicates that neurons and endocrine cells do have a common metabolic need for this isoenzyme. The presence of NSE in APUD cells provides a powerful marker for the discovery of new cell types of the APUD series. Such cells have already been presumptively identified in lung, testes and gastrointestinal tract.

Major collaborative relationships have been established with Dr. Schengrund at Hershey Medical School and Dr. Barker of NINCDS in order to study NSE in primary neuronal cell cultures. Levels of NSE have been established in neuronal cultures derived from embryonic rat cerebral cortex and spinal cord. Studies are underway assessing the metabolic state of cultured neurons using NSE levels as a criteria for ATP generating capacity.

Studies using cultured neurons infected with the temperature sensitive Rous Sarcoma virus are also underway in collaboration with Dr. Greg Giotti of the Salk Institute. Carcinogenesis can be induced in these cells by simply changing their incubation temperature. Using NNE and NSE levels the state of neuronal differentiation can be assessed during the carcinogenic process. Such studiesmay provide insights concerning the molecular events associated with the transformation of a neuron to a cancer cell.

Studies of the diurnal profile of NNE and NSE in the pineal gland is also underway in collaboration with Dr. Andrew Parfitt of the AFRI. Since NSE is present in the APUD pinealocyte and the pinealocyte undergoes a metabolic stimulation in the dark, the levels of NSE during light and dark periods might be expected to fluctuate accordingly. Preliminary results have shown a decrease by 1/2 in pineal NSE levels during the day. Maintenance of animals in constant light is expected to produce a further decrease in NSE levels.

A common thread running through most of the above described studies involves an association between NSE levels and metabolic activity. Changes in NSE levels correlated to increased neuronal activity could prove to be of enormous utility in the study of brain function. The parallel assessment of NNE levels provides the ideal control in all experiments and enables us to appraise neuronal and glial activity simultaneously.

Publications: Z01 MH 01831-02 CP

Marangos, P.J., Parma, A.M., and Goodwin, F.K.: Functional properties of neuronal and glial isoenzymes of brain enolase. J. Neurochem., in press.

Marangos, P.J., Schmechel, D., and Zis, A.P.: The existence and neurobiological significance of neuronal and glial forms of the glycolytic enzyme enolase. J. Biol. Psychiat., in press.

Marangos, P.J., Goodwin, F.K., Parma, A., Lauter, C., and Trams, E.: Neuron specific protein (NSP) in neuroblastoma cells: relation to differentiation. Brain Res. 145:49-58, 1978.

Schmechel, D., Marangos, P.J., Zis, A.P., Brightman, M., and Goodwin, F.K.: The brain enolases as specific markers of neuronal and glial cells. Science 199:313-315, 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 01833-02 CP

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)
Brain Specific Antigens in Neurologic and Psychiatric Disorders

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Paul Marangos	Chief, Unit on Neurochemistry	CP/NIMH
OTHER:	F.K. Goodwin	Chief	CP/NIMH
	Irl Extein	Clinical Associate	CP/NIMH
	Frank Majors	Clinical Associate	CNB/NIMH
	Barry Festoff	Chief of Neurology	VAH/KC
	Mike Ebert	Chief, Section on Experimental Therapeutics	LCS/NIMH
	King Engel	Chief	MNB/NINCDS

COOPERATING UNITS (if any)
Clinical Neuropharmacology Branch, NIMH; Veterans Administration Hospital, Kansas City, Mo.; Laboratory of Clinical Science, NIMH; Medical Neurology Branch, NINCDS

LAB/BRANCH
Clinical Psychobiology Branch

SECTION

INSTITUTE AND LOCATION
NIMH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.7	0.5	

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Various neurological disorders such as Huntington's chorea, Alzeimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, stroke and multiple sclerosis are known to involve neuronal and/or glial cytopathology. The availability in our laboratory of specific radioimmunoassays (RIA) for neuron specific enolase (NSE) and for non-neuronal enolase (NNE) enables us to determine the levels of these two proteins in serum and CSF of neurologic patients. The results of such studies may be of considerable diagnostic value. Studies are also underway in search of antibodies to either NNE or NSE in serum of patients. Demonstrations of circulating levels of antibodies directed against components of either neurons or glia in neurologic or psychiatric patients would support an autoimmune mechanism for the particular disorder.

PHS-6040
(Rev. 10-76)

Project Description: Z01 MH 01833-02 CP

Work in our laboratory has established the existence of two isoenzymes of the glycolytic enzyme enolase which are unique in neurobiology in that they are strictly segregated in their cellular localization. Neuron specific enolase (NSE) is only found in neurons and non-neuronal enolase (NNE) is strictly glial within the nervous system. Both of these proteins have been purified to homogeneity, structurally characterized and specific radio-immunoassays (RIA) developed for each. Very recently NNE and NSE have been isolated from human brain and the RIA's developed for each.

The availability of an efficient, sensitive means for quantitating these neuronal and glial components in human tissues makes clinical studies possible. The clinical studies currently in progress involve the determination of NSE and NNE levels in the serum and CSF of neurological and psychiatric patient groups and their comparison to those of normal controls. The conceptual basis for such a project resides in the hypothesis that diseases having predominantly a neuronal pathology might be characterized by an efflux of NSE into the CSF, while diseases with primary glial pathology would show an NNE efflux. Collaborative studies are currently in the initial stages in attempts to obtain serum and CSF from controlled patient populations. Projects are beginning with Huntington's chorea, amyothrophic lateral sclerosis, Alzheimer 's disease, Parkinson's disease, multiple sclerosis and stroke patients. A large group of normal CSF samples have been assayed and very low levels of both NNE and NSE are present ($\simeq$ 10 ng/ml). Known amounts of pure NNE and NSE added to normal CSF yield the predicted assay values clearly indicating that both antigens can be reliably measured in this fluid.

If elevated CSF and serum levels of NSE are found in various disorders we will assay for the presence of the anti-NSE immunoglobulin. Such a procedure is easily executed by simply adding labelled NSE to patient serum and assaying for IgG bound radioactivity. Such a study would represent a direct test of the autoimmune concept of neurological disease. The autoimmune hypothesis, generally stated, contends that some primary lesions lead to the body making antibodies directed against its own cellular components.

The presence of neuronal and/or glial enolase in CSF or serum of neurologic or psychiatric patient subgroups would be highly significant from a diagnostic standpoint, as it would represent a relatively non-invasive means of assessing specific cellular damage. The procedure may be of value for assessing the severity of strokes or cardiac arrests where brain cell damage is expected to occur.

Publications: Z01 MH 01833-02 CP

Marangos, P.J., Zis. A.P., Clark, R.L. and Goodwin, F.K.: Neuronal, non-neuronal and hybrid forms of enolase in brain. Brain Res., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 01834-01 CP

PERIOD COVERED

October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Studies on Endogenous Ligands Specifically Interacting with Benzodiazepine Receptors in Brain

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Paul J. Marangos	Chief, Unit on Neurochemistry	CP/NIMH
OTHER:	Philip Skolnick	Pharmacologist	LPS/NIAAA
	Steven Paul	Clinical Associate	CP /NIMH
	Frederick K. Goodwin	Chief	CP/NIMH
	Leonard Cook	Director of Pharmacol. Res.	Hoffman-LaRoche
	Arnold Davidson	Director Drug Res.	Hoffman-LaRoche
	Jeffery Barker	Medical Officer	LNP/NINCDS

COOPERATING UNITS (if any)

Laboratory of Clinical Science, NIMH; Laboratory of Preclinical Studies, NIAAA; Hoffmann LaRoche, New York; Laboratory of Neuropharmacology, NINCDS.

LAB/BRANCH

Clinical Psychobiology Branch

SECTION

INSTITUTE AND LOCATION

NIMH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.7	0.4	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The recent demonstration of benzodiazepine specific receptors in brain suggests that at least some of the behavioral and physiological effects of these tranquilizer drugs are mediated through a specific receptor. These recent observations have prompted the search for endogenous ligands in brain that specifically interact with the benzodiazepine receptor, with the goal of elucidating and characterizing an anxiety mediating system. Studies thus far conducted have led to the isolation and identification of the two purine derivatives, inosine and hypoxanthine that competitively inhibit ^{3}H diazepam binding to rat brain synaptic membranes. In vivo pharmacological studies are in progress aimed at determining whether inosine and hypoxanthine represent the physiologically relevant endogenous ligands for the benzodiazepine receptors. Recently it has been suggested that a specific protein may bind the benzodiazepine receptor and we have begun work on its isolation and characterization. Future work on this protein coupled with further characterization of inosine and hypoxanthine binding should provide considerable insight concerning anxiety mediating systems in brain.

Project Description: Z01 MH 01834-01 CP

Anxiety may be viewed as an underlying component in many psychiatric disorders. Drugs such as the benzodiazepines (Valium, Librium) have been shown to be anxiolytic agents in humans and are widely used for their anxiolytic, hypnotic and muscle relaxant properties.

The very recent discovery of a specific receptor for diazepam in rat and human brain tissue has elicited considerable speculation concerning the existence of an endogenous ligand with the specific ability to bind and stimulate this receptor. The evidence indicates that the pharmacological properties of diazepam are receptor mediated and the key question remains as to what agents in brain are capable of interacting with the receptor. Identification and characterization of the proposed endogenous ligand would likely reveal the nature of this system in brain and increase our understanding of the mechanism by which the brain regulates anxiety, muscle tension and spasmodic activity.

During the last six months we have isolated from bovine brain tissue two compounds with the ability to specifically and competitively inhibit the binding of ^{3}H diazepam to rat brain synaptic membranes. Extensive physical-clinical analysis using gel filtratives, ion exchange chromatography, high pressure liquid chromatography, thin layer chromatography and spectrophotometric analysis has established that the two compounds able to interact with benzodiazepine receptor are the purines, inosine and hypoxanthine. Micromolar concentrations are required for receptor interactions indicating that the receptor affinity of each is very low. However, brain levels of inosine and hypoxanthine have been reported in this range. The physiological significance of inosine and hypoxanthines in vitro interaction with the benzodiazepine receptor is still unclear. Studies are in progress aimed at determining whether these two compounds have "benzodiazepine like" pharmacologic properties in tissue culture and whole animals. The results of such studies will determine whether inosine and hypoxanthine are in fact the physiologically relevant endogenous ligands for the benzodiazepine receptors.

It has very recently been demonstrated that a protein exists in brain that when incubated with rat brain synaptic membranes inhibits the binding of ^{3}H diazepam. We have undertaken the isolation of this protein from bovine brain. This project is only in the pilot stage at this time but the results indicate that the protein is small, stable to heat and acidic in nature. The isolation and characterization of this apparent receptor binding protein will further elucidate the nature of the proposed anxiety mediating system in brain.

Publications: Z01 MH 01834-01 CP

Marangos, P.J., Paul, S.M., Greenlaw, P., Goodwin, F.K., and Skolnick, P.: Demonstration of an endogenous, competitive inhibitor(s) of [^{3}H] binding in bovine brain. Life Sci. 22:1893-1900, 1978.

Skolnick, P., Marangos, P.J., Goodwin, F.K., Edwards, M., and Paul, S.: Identification of inosine and hypoxanthine as endogenous inhibitors of [^{3}H] diazepam binding in the central nervous system. Life Sci., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01835-01 CP

PERIOD COVERED
October 1, 1977 - September 30, 1978

TITLE OF PROJECT (80 characters or less)
Behavioral and Biochemical Effects of Endorphin Agonists and Antagonists in Animals and Man

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Frederick Goodwin	Chief	CP/NIMH
OTHER:	Paul Marangos	Chief, Unit on Neurochemistry	CP/NIMH
	Irl Extein	Clinical Associate	CP/NIMH
	Alfred Lewy	Staff Psychiatrist	CP/NIMH
	Ron Schoenfeld	Guest Scientist	CP/NIMH
	Philip Gold	Chief, Unit on Neuroendocrin.	CP/NIMH
	William Bunney	Chief	BP/NIMH
	Glen Davis	Staff Psychiatrist	BP/NIMH
	Jim Ballenger	Staff Psychiatrist	BP/NIMH
	Mark Gold	Psychiatric Resident	Yale U.
	Eugene Redmond	Assoc. Prof. of Psychiatry	Yale U.
	Gregory Brown	Chief, Dept. of Neuroscience	McMaster U.
	Choh Hao Li	Hormone Research Lab.	U.C.S.F.

COOPERATING UNITS (if any)
Biological Psychiatry Branch, NIMH; Yale University; McMaster University; University of California, San Francisco.

LAB/BRANCH
Clinical Psychobiology Branch

SECTION

INSTITUTE AND LOCATION
NIMH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.8	0.4	

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords) We are interested in the role of endogenous opiate peptides, called endorphins, in central nervous system functioning in general and in mental illness in particular. Much of our work has centered around FK 33-824, a synthetic enkephalin analogue which can be administered parenterally and orally and hence may be of use clinically. Animal studies have included demonstration of FK 33-824 inhibition of conditioned avoidance responding in rats and blockade of anxiety behavior and increase in serum prolactin in monkeys. These results suggest an interaction of FK 33-824 with brain dopamine and norepinephrine systems and possible antipsychotic and antianxiety properties in man. In patients with affective disorder and schizophrenia a double-blind placebo controlled trial of beta-endorphin is underway, and a similar trial of FK 33-824 is planned. Another strategy for identifying the role of the endorphin system is to study the effects of the opiate antagonist naloxone. We are measuring the neuroendocrine effects of naloxone in volunteers, and have shown little effect of naloxone in mania.

PHS-6040
(Rev. 10-76)

Endorphins are endogenous opiate peptides found in the brain. There is a growing body of laboratory and clinical evidence that endorphins play a role in important CNS functions, including pain perception, mood, behavior, and neuroendocrine functioning. Much of our work has involved the use of FK 33-824, an analogue of the naturally occurring pentapeptide methanine enkephalin, synthesized by Sandoz, Ltd. Because FK 33-824, in contrast to the natural endorphins, can be synthesized and administered with relative ease, it is a drug which might be of use clinically. It has been administered safely to volunteers in Europe.

We have been investigating the pharmacology of the endorphin system in a number of ways, including behavioral and biochemical studies of endorphin agonists in animals and clinical and neuroendocrine studies of endorphin agonists and antagonists in humans. Studies of the behavioral effects of FK 33-824 in animals include: 1) comparison of FK 33-824 induced catatonia in rats to that produced by morphine and haloperidol; 2) observation in rats of behavioral effects of FK 33-824, including its effects on dopamine mediated stereotyped behavior induced by apomorphine and amphetamine; 3) inhibition by FK 33-824 of conditioned avoidance responding in rats; 4) blockade by FK 33-824 in monkeys of piperoxan - induced anxiety behavior, which is mediated via activation of the noradrenergic locus coeruleus in the brain (in collaboration with Drs. Gold and Redmond at Yale). Biochemical studies of FK 33-824 include: 1) measurement of amine metabolites in monkey CSF; 2) demonstration of an increase in serum prolactin in monkeys after FK 33-824. The inhibition of conditioned avoidance and elevation of prolactin are consistent with an antagonistic effect of FK 33-824 on dopamine systems and suggest possible antipsychotic efficacy for the compound.

There is a preliminary report of improvement in a variety of major psychiatric disorders after administration of beta-endorphin. Following up on this, we are collaborating with the Biological Psychiatry Branch of the NIMH in a double-blind placebo controlled study of the effects of beta endorphin administered intravenously to patients with affective disorder and schizophrenia. The beta-endorphin is being supplied by Dr. Li. In addition to intensive observation of mental status, the effects on pain sensitivity and neuroendocrine parameters are being measured. A similar clinical trial of FK 33-824 is planned pending receipt of an IND from the FDA.

We have administered the specific opiate antagonist naloxone in humans. Presumably, changes produced by naloxone administration involve the endorphin system. In order to elucidate the role of endorphins in neuroendocrine regulation, naloxone (20 mg) has been injected s.c. in male volunteers and serial blood samples taken for assay of a variety of hypothalamic hormones. A similar study in female volunteers is planned, as is a study of naloxone's effects on a TRH infusion.

Following up on a report that naloxone caused improvement in a subset of manic patients, we collaborated with BPB in administering naloxone 20 mg. s.c. to ten manic patients with a placebo controlled, double-blind design. In only one of the ten patients was any positive effect observed.

Publications: ZO1 MH 01835-01

Extein, I., Goodwin, F.K., Lewy, A.J., Schoenfeld, R.I., Fakhuri, L.R., Gold, M.S., and Redmond, D.E., Jr.: Behavioral and biochemical effects of FK 33-824, a parenterally and orally active enkephalin analogue. In Endorphins in Mental Health Research. Usdin, E. (Ed.), McMillan, London, 1978, (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00421-16 LCS

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)
Neuroanatomy and Metabolism of Biogenic Amines in the Central Nervous System

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. Saavedra, Visiting Scientist	LCS NIMH
Other:	J. Axelrod, Chief, Section on Pharmacology	LCS NIMH
	P. Settler	SKF Labs
	R. McCarty, Research Associate	LCS NIMH
	W. Oertel, Guest Worker	LCS NIMH
	P. Guicheney, Guest Worker	LCS NIMH

COOPERATING UNITS (if any)
Smith, Kline & French Laboratories: Philadelphia, Pa.

LAB/BRANCH
Laboratory of Clinical Science

SECTION
Section on Pharmacology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	1.5	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Acute immobilization stress results in a decrease in adrenaline levels in brain stem nuclei. These changes occur in brain stem regions probably related to the changes observed in spontaneously hypertensive rats. Adrenaline changes were more pronounced and widespread than those on noradrenaline, indicating that those two catecholamines may have different roles regarding the cardiovascular regulatory mechanisms and the response to stress. Serotonin and histamine appear to be concentrated in areas of the brain stem where changes in catecholamines occur during hypertension.

PHS-6040
(Rev. 10-76)

During the period covered this project was incorporated in Project No. Z01 MH 00425-02 LCS. Please refer to that project for information and publications.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00422-07 LCS

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Biochemical and Pharmacological Studies on the Pineal Gland

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. Zatz, Senior Staff Fellow	LCS NIMH
Other:	M. Weinstock, Guest Worker	LCS NIMH
	M. Brownstein, Medical Officer (Research)	LCS NIMH
	J. Axelrod, Chief, Section on Pharmacology	LCS NIMH

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Clinical Science

SECTION
Section on Pharmacology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.3	1.3	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project was initiated to use the rat pineal gland as a model system in which to study the regulation of circadian rhythms and the role of cyclic nucleotides in neurotransmission.

Cyclic GMP is released from the sympathetic nerve endings in the rat pineal gland in association with exocytosis. Lithium inhibits the response of both cyclic AMP and cyclic GMP to stimulalation. Elevated nocturnal levels of N-acetyltransferase require continued activity of the suprachiasmatic nucleus of the hypothalamus. Central depressants can cause a rapid fall in enzyme activity. Light and carbachol both can reduce enzyme activity and reset the circadian mechanism resulting in a phase shift of the pineal rhythm.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: To use the pineal as a model to learn how circadian rhythms are generated and regulated and to study the roles of cyclic nucleotides.

Methods Employed: Biochemical, anatomical, pharmacological, and radioactive tracer techniques.

Major Findings: Cyclic GMP is released from the sympathetic nerve endings in the rat pineal gland in association with exocytosis of catecholamine. Depolarization by potassium or veratridine, but not tyramine, promotes the release of cyclic GMP. Electric field stimulation also causes release of cyclic GMP, which is dependent on the presence of extracellular calcium and intact nerve endings. The cyclic nucleotide is not, itself, released by exocytosis, however, since its efflux is blocked by probenecid, an inhibitor of acid transport.

Low concentrations of lithium inhibit the generation of cyclic AMP and of cyclic GMP in response to stimulation. Lithium interferes with the effects of magnesium on hormone-sensitive adenylate cyclase. There may also be an inhibition of the effects of magnesium on binding of agonists to the ß-adrenergic receptor. A specific effect of lithium on the regulation of sensitivity has not been found.

Maintenance of the elevated nocturnal levels of pineal serotonin N-acetyltransferase requires continued activity of the suprachiasmatic nucleus which ultimately results in increased release of norepinephrine from the sympathetic nerve endings in the gland. Central depressants can interfere with transmission of the output, resulting in a rapid fall in enzyme activity. They have not been shown to act directly on the oscillatory mechanism. In contrast, light and carbachol can reduce nocturnal enzyme activity by a direct effect on the circadian oscillator. Each can reset the circadian mechanism resulting in a phase shift of the pineal rhythm. These data demonstrate the feasibility of a neuropharmacologic approach to the mechanisms regulating circadian rhythms in mammals.

Significance to Biomedical Research: Circadian rhythms occur in hormone levels, activity, mood, and pineal as well as other physiological functions. Elucidation of the mechanisms generating or regulating such rhythms is of broad biological interest. The role of cyclic nucleotides in pre- and postsynaptic functions should provide a better understanding of the regulation of neurotransmitter release and function.

Proposed Course of Project: The mechanisms generating and regulating circadian rhythms will be explored further.

Publications:

Axelrod, J. and Zatz, M.: The ß-adrenergic Receptor and the Regulation of Circadian Rhythms in the Pineal Gland. In Litwack, G. (Ed.): Biochemical Actions of Hormones. New York, Academic Press, Inc., 1977, Volume IV, Chapter 5, pp. 249-268.

Kebabian, J.W., Zatz, M. and O'Dea, R.F.: Modulation of Receptor Sensitivity in the Pineal: The Roles of Cyclic Nucleotides. In Cowan, W.M. and Ferrendelli, J.A. (Eds.): Neuroscience Symposia. Bethesda, Society for Neuroscience, 1977, Volume II, pp. 376-398.

Zatz, M.: Effects of cholera toxin on supersensitive and subsensitive rat pineal glands: regulation of sensitivity at multiple sites. Life Sci., 21: 1267-1276, 1977.

Zatz, M. and Weinstock, M.: Electric field stimulation releases norepinephrine and cyclic GMP from the rat pineal gland. Life Sci., 22: 767-772, 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00423-05 LCS

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)
Studies on Protein Carboxymethylation

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: C. Gagnon, Guest Worker — LCS NIMH
W. Strittmatter, Staff Fellow — LCS NIMH
J. Axelrod, Chief, Section on Pharmacology — LCS NIMH

Other: W. Bardin, Professor of Medicine, Hershey Medical Center

COOPERATING UNITS (if any)
Department of Medicine; Hershey Medical Center, Hershey, Pa.

LAB/BRANCH
Laboratory of Clinical Science

SECTION
Section on Pharmacology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	2.0	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The administration of isoproterenol causes a marked increase in the activity of protein carboxymethylase and its acceptor protein in rat parotid glands. This stimulation of protein carboxymethylase has been shown to be mediated by a ß adrenergic receptor. Stimulation of the posterior pituitary by the ingestion of sodium chloride caused an increase in protein carboxymethylase in the posterior pituitary and a decrease of an acceptor protein tentatively identified as neurophysin. Protein carboxymethylase has been found to be highly localized in sperm of rat. In an infertile rat mutant the activity of protein carboxymethylase is markedly diminished.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: To study the properties, distribution and physiological role of protein carboxymethyltransferase and its acceptor proteins.

Methods Employed: Enzymatic, radiometric, and endocrinological procedures.

Major Findings: Stimulation of the rat posterior pituitary by the ingestion of sodium chloride resulted in an increase in protein carboxymethylase. There was also a marked decrease in an acceptor protein in the pituitary which was tentatively identified as neurophysin. Protein carboxymethylase has been found to be highly localized in the sperm of the rat. The activity of protein carboxymethylase was markedly diminished in an infertile rat mutant.

The administration of isoproterenol caused a considerable increase in the activity of protein carboxymethylase and in its acceptor protein(s) in rat parotid gland. This stimulatory activity has the properties of a ß adrenergic receptor.

Significance to Biomedical Research: Protein carboxymethylase and its acceptor protein(s) appear to be important in the action of secretory organs and the reproductive process.

Proposed Course of Project: The role of protein carboxymethylase in secretion and reproduction will be further explored.

Publications:

Gagnon, C., Viveros, O.H., Diliberto, E.J., Jr. and Axelrod, J.: Enzymatic methylation of carboxyl groups of chromaffin granule membrane proteins. J. Biol. Chem. 253: 3778-3781, 1978.

O'Dea, R.F., Viveros, O.H., Acheson, A., Gorman, C. and Axelrod, J.: Protein carboxymethylase and methyl-acceptor proteins in human platelets and erythrocytes. Biochem. Pharmacol. 27: 679-684, 1978.

O'Dea, R.F., Viveros, O.H., Axelrod, J., Aswankikumar, S., Schiffmann, E., Corcoran, B.A.: Rapid stimulation of protein carboxymethylation in leukocytes by a chemotactic peptide. Nature 272: 462-464, 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00424-03 LCS

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Biologically Active Peptides in the Brain

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. Brownstein, Medical Officer (Research)	LCS NIMH
	J. Schaeffer, Staff Fellow	LCS NIMH
Other:	H. Gainer, Functional Neurochemical Sec.Chief	LDN NICHHD
	D. Fink	LDN NICHHD
	J. Russell	LDN NICHHD
	S. Leeman, Asst. Prof. Physiol.; Harvard Medical School	
	E. Mroz, Post Doctoral Fellow, Harvard Medical School	
	D. Krieger, Prof. Med., Mt. Sinai School of Med., NYC	
	A. Liotta, Dept. Med., Mt. Sinai School of Med., NYC	
	J. Roth, Diabetes Branch Chief	IRP NIAMDD
	J. Havrankova, Diabetes Branch	IRP NIAMDD
	D. Schmeckel, Medical Officer	LCS NIMH

COOPERATING UNITS (if any)
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Mt. Sinai School of Medicine

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Section on Pharmacology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH,Bethesda, Maryland 20014

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2.0	2.0	0.0

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Proteins synthesized, transported and stored by neurons of the hypothalamo-neurohypophysial system have been partially characterized. Insulin receptors, and insulin itself, have been shown to be present in the brain of the rat.

ACTH, ß-endorphin, and ß-lipotropin have been measured in the brain after a variety of physiological manipulations and preliminary studies of the biosynthesis of these hormones by brain have been conducted.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: To locate specific groups of neurons in the central nervous system which synthesize and secrete peptides; to determine the biochemical mechanisms underlying the synthesis of peptides by neurosecretory cells; to study interactions between peptidergic and non-peptidergic neurons.

Methods Employed: Biosynthetic, enzymatic and histologic procedures; microdissection techniques; immunocytochemistry.

Major Findings: Studies have continued on the biosynthesis of the two major neurophysins found in rat. Efforts are currently being directed towards characterizing the large precursors which give rise to the neurophysins and isolating the enzymes involved in metabolizing these precursors. Studies have been initiated on the synthesis and "natural history" of the slowly transported protein species found in the hypothalamo-neurohypophysial system. It is planned to characterize these proteins and to study alterations in their rates of synthesis and transport that accompany physiological manipulation. An attempt to characterize the TRH-like substance in the retina of the rat is in process and it is hoped that the study of the biosynthesis of TRH in retinal tissue will be possible. In addition, dissociation of the retina and the separation of the dissociated cells on density gradients has been accomplished. It is hoped to study the neurochemistry of the isolated cells.

ACTH and the opioid peptides in the CNS are being studied. Changes in the levels of these hormones in the CNS do not parallel changes in their peripheral levels. In addition to examining the steady-state levels of ACTH, ß-endorphin, and ß-lipotropin, the biosynthesis of these compounds in the CNS is being examined.

It has been shown that brain membranes bind insulin with marked specificity and high affinity. The insulin "receptors" are essentially identical to those found in the periphery and are heterogeneously distributed in the CNS. In addition to receptors, there is also insulin in the brain. The insulin levels are far in excess of blood levels. The origin of this insulin remains to be determined, but neuronal perikarya have been demonstrated immunocytochemically which seem to contain insulin.

Significance to Biomedical Research: Peptides secreted by neurons in the CNS appear to act not only as factors which control the anterior pituitary but also as agents (neurotransmitters) which allow nerve cells to interact with one another. Thus it is important to develop methods for studying these compounds.

Proposed Course of Project: The effect of endocrinological, surgical and pharmacological manipulations on peptide secretion will be studied.

Publications:

Krieger, D.T., Liotta, A., Suda, T., Palkovits, M. and Brownstein, M.J.: Presence of immunoassayable ß-lipotropin in bovine brain and spinal cord: lack of concordance with ACTH concentrations. Biochem. Biophys. Res. Commun. 76: 930-936, 1977.

Palkovits, M., Saavedra, J.M., Jacobowitz, D.M., Kizer, J.S., Zaborsky, L. and Brownstein, M.J.: Serotonergic innervation of the forebrain: effects of lesions on serotonin and tryptophan hydroxylase levels. Brain Res. 130: 121-134, 1977.

Brownstein, M.J.: Studies of the Distribution of Biologically Active Peptides in the Brain. In Porter, J.C. (Ed.): Hypothalamic Peptide Hormones and Pituitary Regulation. New York, Plenum Publishing Co., 1977, pp. 41-48.

Brownstein, M.J.: Peptides in Mammalian Neural Tissue. In Lipton, M.A., DiMascio, A. and Killam, K.P. (Eds.): Psychopharmacology: A Generation of Progress. New York, Raven Press, 1978, pp. 397-401.

Havrankova, J., Brownstein, J.J. and Roth, J.: Insulin receptors are widely but unevenly distributed throughout the central nervous system of the rat. Nature 272: 827-829, 1978.

Gagnon, C., Axelrod, J. and Brownstein, M.J.: Protein carboxymethylation: effects of 2% sodium chloride administration on protein carboxymethylase and its endogenous substrates in rat posterior pituitary. Life Sci. (in press).

Gainer, H. and Brownstein, M.J.: Electrophoretic analyses of proteins transported to the rat posterior pituitary. J. Neurochem. (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00425-02 LCS

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)
Peripheral and Central Catecholamines in Hypertension and Stress

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. Saavedra, Visiting Scientist	LCS NIMH
Other:	R. Kvetnansky, Visiting Scientist	LCS NIMH
	J. Kebabian, Experimental Therapeutics Br.	NINCDS

COOPERATING UNITS (if any)

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Section on Pharmacology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.5	0.5

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project was initiated to study the role of catecholamines in the central and peripheral nervous system in experimental and genetic hypertension. Increased adrenaline formation has been demonstrated in specific brain areas of spontaneously hypertensive and doca-salt hypertensive rats. These changes coincide with changes in the formation of adrenaline in the adrenal medulla. Administration of inhibitors of adrenaline synthesis resulted in a normalization of the high blood pressure.

During stress, similar changes in brain adrenaline are found in specific areas of the brain stem and hypothalamus. These findings suggest that localized areas in the rat brain and specific brain adrenergic neurons can play a role in the development of hypertension, in the stress reaction, and in the regulation of adrenomedullary catecholamine metabolism.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: To study the role of peripheral and central catecholamines in the central and peripheral nervous system in experimental and genetic hypertension and stress.

Methods Employed: Microdissection of brain nuclei; selective lesions on brain nuclei by specific neurotoxins; enzymatic-isotopic micromethods for the measurement of biogenic amines and related enzymes.

Major Findings: In young (4-week-old) spontaneously hypertensive rats (SHR), adrenaline levels are decreased in areas of the brain with high adrenaline-forming enzyme (PNMT) activity, suggesting an increased adrenaline turnover. These changes coincide in time with decreased activities of the adrenaline-forming enzymes in the adrenal medulla.

In adult SHR the changes in brain adrenaline are no longer present, and increased activity of the adrenomedullary catecholamine-forming enzymes occurs.

The administration of a non-α-blocker, PNMT inhibitor, resulted in a normalization of the high blood pressure in the SHR, suggesting that central and/or peripheral adrenergic mechanisms can be related to the onset and/or maintenance of the hypertension. Hypertensive (Doca-salt) rats, produced experimentally, show similar brain changes in adrenaline levels and PNMT activity, localized to the same brain stem areas as the SHR. These changes are more widespread after a long treatment with DOCA and salt (10 weeks) which results in a large increase in the blood pressure.

In neurogenic hypertensive rats (bilateral sino-aortic denervation), preliminary results indicate that PNMT activity is also increased in the brain stem (nucleus tractus solitarius area).

Acute immobilization stress results in a decrease in adrenaline levels in brain stem nuclei. These changes occur in brain stem regions probably related to the changes overserve in SHR. Adrenaline changes were more pronounced and widespread than those on noradrenaline, indicating that those two catecholamines may have different roles regarding the cardiovascular regulatory mechanisms and the response to stress. Adrenaline levels were also decreased in selective hypothalamic areas indicating that this amine, besides noradrenaline and dopamine, is also involved in the stress reaction.

A neuroanatomical and biochemical study on dopamine neurons in the substantia nigra was performed. A selective lesion of the

dopaminergic neurons in that area resulted in increases in glutamic acid decarboxylase activity, indicating the possibility of a physiological interaction between gabaergic and dopaminergic systems.

The nigral dopamine-sensitive adenylate cyclase was not shown to be localized in the dopaminergic neurons.

A detailed neuroanatomical study of the serotoninergic innervation of the rat brain after different brain lesions was performed.

The nerve growth factor (NGF) was shown to stimulate PNMT activity and serotonin formation in the superior cervical ganglion.

Significance to Biomedical Research: There are selective changes in catecholamine (adrenergic and noradrenergic) neurons in specific brain areas in spontaneous and experimental hypertension. These findings might explain the action of antihypertensive drugs like clinidine and α-methyl dopa, by stimulation of hypothalamic α-adrenergic receptors.

The administration of inhibitors of the adrenaline-forming enzyme (PNMT) results in a normalization of the high blood pressure in both experimental and genetic hypertension, and can be used as a tool to explore the contribution of central and peripheral catecholamines to the regulation of blood pressure, the stress reaction, and the control of the function of the adrenal medulla.

The combination of microanatomical and microchemical techniques allows us to study changes on neurotransmitter metabolism in over 100 selective brain areas.

Proposed Course of Project: Catecholamines and related enzymes will be examined in specific brain nuclei in various forms of hypertension (neurogenic, salt-sensitive genetic, renal) during the development of the hypertension and after pharmacological manipulations. The inter-relation between brain catecholamines, other biogenic amines (histamine, serotonin) and neuropeptides in hypertension and stress will be examined.

Publications:

Grobecker, H., Saavedra, J.M., Roizen, M.F., Weise, V., Kopin, I.J. and Axelrod, J.: Peripheral and central catecholaminergic neurons in genetic and experimental hypertension in rats. Clin. Sci. Mol. Med. 51: 377s-380s, 1976.

Saavedra, J.M., Grobecker, H. and Axelrod, J.: Adrenaline-forming enzyme in brain stem: elevation in genetic and experimental hypertension. Science 191: 483-484, 1976.

Publications (cont.):

Liuzzi, A., Foppen, F.H., Saavedra, J.M., Jacobowitz, D., and Kopin, I.J.: Effect of NGF and dexamethasone on phenylethanolamine-N-methyltransferase (PNMT) activity in neonatal rat superior cervical ganglia. J. Neurochem. 28: 1215-1220, 1977.

Liuzzi, A., Foppen, F.H., Saavedra, J.M., Levi-Montalchini, R. and Kopin, I.J.: Gas chromatographic-mass spectrometric assay of serotonin in rat superior cervical ganglia. Effects of nerve growth factor and 6-hydroxydopamine. Brain Res. 133: 354-357, 1977.

Saavedra, J.M.: Distribution of serotonin and synthesizing enzymes in discrete areas of the brain. Fed. Proc. 36: 2134-2141, 1977.

Saavedra, J.M.: Microassay of Biogenic Amines in Neurons of Aplysia. The Coexistence of More than One Transmitter Molecule in a Neuron. In Osborne, N. (Ed.): Biochemistry of Characterized Neurons. New York, Pergamon Press, 1977, pp. 217-238.

Saavedra, J.M.: ß-Phenylethylamine: Is this Biogenic Amine Related to Neuropsychiatric Diseases? In Moshaim, A. and Wolf, M. (Eds.): Modern Pharmacology-Toxicology. New York, Marcel Dekker, Inc., 1978, Vol. 12, Part 1, pp. 139-157.

Saavedra, J.M.: Tryptamine and Dimethyltryptamine: Are They Related to Psychiatric Disorders? In Usdin, E., Hamburg, D. and Barchase, J. (Eds.): Neuroregulators and Psychiatric Disorders. New York, Oxford University Press, 1977, pp. 201-209.

Saavedra, J.M., Grobecker, H., and Axelrod, J.: Central adrenergic neurons in spontaneous (SHR) and experimental hypertension in rats. Clin. Exper. Pharmacol. Physiol. Suppl. 3: 157-160, 1977.

Axelrod, J.: Central Catecholamine Neurotransmitters and Psychoactive Drugs. In van Praag, H. and Bruinvels, J. (Eds.): Neurotransmission and Disturbed Behavior. Utrecht, Bohn, Scheltema and Holkema, 1977, pp. 6-18.

Axelrod, J.: Introduction: Mesocortical Dopaminergic Neurons. In Costa, E. and Gessa, G. (Eds.): Advances in Biochemical Psychopharmacology. New York, Raven Press, 1977, Vol. 16, pp. 1-3.

Axelrod, J.: Catecholaminergic systems in brain. Acta Neurol. Scand. 56: 85-89, 1977.

Publications (cont.)

Axelrod, J.: Catecholamines: Effects of ACTH and adrenal corticoids. Annals N.Y. Acad. Sci. 297: 275-283, 1977.

Dratman, M.B., Crutchfield, F.L., Axelrod, J., Utiger, R.D. and Marsh, E.: Triiodothyronine in rat submaxillary salivary gland: Effects of altered adrenergic function. Pharmacology 51: 193-207, 1977.

Kebabian, J.W.: Cyclic Nucleotides and Synaptic Transmission in Sympathetic Ganglia. In Costa, E. and Gessa, G. (Eds.): Advances in Biochemical Psychopharmacology. New York, Raven Press, 1977, Vol. 16, pp. 533-539.

Saavedra, J.M., Grobecker, H. and Axelrod, J.: Changes in central catecholaminergic neurons in the spontaneously (genetic) hypertensive rat. Cir. Res. 42: 529-534, 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00426-02 LCS

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Catechol Estrogens: Localization, Synthesis, Metabolism and Effects in Estrogen-Sensitive Tissues

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: S. Paul, Staff Fellow — LCS NIMH
J. Axelrod, Chief, Section on Pharmacology — LCS NIMH
M. Lippman, Medicine Branch — NCI

COOPERATING UNITS (if any)

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Laboratory of Clinical Science

SECTION
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NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	0.0

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Catecholestrogens were found to have a high affinity for estrogen receptors of brain, uterus, mammary tissues and liver. Preliminary results using a mammary carcinoma cell line in vitro indicate that catecholestrogens have an inhibiting effect on cell growth. Catecholestrogens can compete with estrogen receptors in these tumors. Both the catechol-forming enzyme and its inactivating enzyme, catechol-O-methyltransferase, are present in human breast tissues. A highly significant increase in catechol-O-methyltransferase activity was found in malignant breast tissue.

Estrogen has been found to release endogenous noradrenaline and dopamine from certain areas of the rat hypothalamus.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: To utilize sensitive radioenzymatic methods to study possible physiological effects of the catechol estrogens.

Methods Used: Enzymatic, endocrinologic, tissue and organ culture.

Major Findings: The catechol estrogens, major metabolites of estrogen in laboratory animals and man, appear to have a high affinity for estrogen receptors of various target tissues including brain, uterus, mammary tissue, and liver. Studies have been initiated to test whether these metabolites have estrogen-like or anti-estrogen effects in various *in vitro* and *in vivo* test systems. Using short-term hypothalamic cultures we have shown that catechol estrogens can function as anti-estrogens in inhibiting estrogen-induced accumulations of cAMP. Preliminary results using a mammary carcinoma cell line *in vitro* (MCF-7) suggest that, at concentrations between 10^{-6} m and 10^{-8} M, $2OHE_2$ has inhibitory effects on cell growth. In these same tumor cells, binding studies using radioactive labelled catechol estrogens have demonstrated these compounds to have an affinity for estrogen receptors that is within one order of magnitude of estradiol-17β.

Since catechol estrogens are present in estrogen-sensitive tissues (Paul and Axelrod, 1977a, 1977b) and since they may play a role in regulating estrogen effects in these tissues (Paul and Skolnick, 1977), studies on their possible role in human breast cancer have been initiated. Both the catechol estrogen-forming enzyme and the major metabolic enzyme, catechol-O-methyltransferase (COMT), have been demonstrated and characterized in human mammary tumor specimens. Although only a small amount of catechol estrogen-forming activity has been observed in the human breast samples, a highly significant increase in COMT activity was found in the malignant breast tumors when compared to both benign tumors or normal breast tissue.

Significance to Biomedical Research: Catechol estrogens have been found to have potent endocrine effects. Preliminary work suggests that they may be involved in estrogen-sensitive tissues. These findings have, therefore, implicated these metabolites in various physiological and(or) pathophysiologic conditions.

Proposed Course of Project: Studies on the two major enzymes involved in the synthesis and metabolism of these compounds will be carried out under various physiological and pharmacological conditions. Development of a plasma assay suitable for human plasma samples will continue, so that the circulating concentration of these metabolites in various normal and disease conditions can be measured. Preliminary tissue culture work will be extended.

Publications:

Paul, S.M. and Axelrod, J.: Catechol estrogens: presence in brain and endocrine tissues. Science 197: 657-659, 1977.

Paul, S.M. and Axelrod, J.: A rapid and sensitive radioenzymatic assay for catechol estrogens in tissues. Life Sci. 21: 493-502, 1977.

Paul, S.M. and Axelrod, J.: Catechol estrogen-forming enzyme of brain: demonstration of a cytochrome P450 monooxygenase. Endocrinology 101: 1604-1610, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00427-01 LCS

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)
Phospholipid Methylation and Membrane Structure and Function

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: F. Hirata, Visiting Scientist LCS NIMH
W. Strittmatter, Research Associate LCS NIMH
J. Axelrod, Chief, Section on Pharmacology LCS NIMH

COOPERATING UNITS (if any)

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NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Two methyltransferase enzymes have been found to synthesize phosphatidyl choline from phosphatidylethanolamine in adrenal medulla and erythrocyte membranes. These enzymes have been found to be asymmetrically distributed in erythrocyte membranes and involved in the translocation of phospholipids from cytoplasmic to the outer surface of membranes. The enzymatic methylation of phosphatidylethanolamine to phosphatidyl N-monomethylethanolamine causes a profound change in membrane fluidity. Stimulation of the ß adrenergic receptor in reticulocyte ghosts causes an increase in methylation fluidity and translocation of phospholipids and coupling of ß adrenergic receptor with adenylate cyclase. More ß adrenergic receptors were found to be available to binding by its ligands when phospholipid methylation is increased by the addition of S adenosyl methionine reticulocyte ghosts.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: To study the role of phospholipid methylation in membrane structure and regulation of ß adrenergic receptor.

Methods Employed: Enzymatic and radiometric.

Major Findings: Two methyl transferases have been found in the adrenal medulla and erythrocyte membranes that synthesize phosphatidylethanolamine. The first enzyme methylates phosphatidylethanolamine to phosphatidyl N monomethylethanolamine, requires Mg^{++} and has a low Km for S-adenosylmethionine. The second enzyme converts the monomethylated phosphatide to phosphatidylcholine. These enzymes have been found to be asymmetrically distributed in erythrocyte membranes and are involved in the translocation of phospholipids from the cytoplasmic to the outer surface of membranes. The product of the first enzyme caused a profound change in membrane fluidity. When the ß adrenergic receptor reticulocyte ghost is stimulated there is an increase in membrane methylation, fluidity and translocation of phospholipids and coupling of the ß adrenergic receptor with adenylate cyclase. When the synthesis of phosphatidylcholine is stimulated by large amounts of S adenosylmethionine more ß adrenergic receptors become available to bind with its ligands.

Significance to Biomedical Research: The new discovery in phospholipidmethylation will provide an opportunity to examine whether there is a defect in these enzymes in membrane diseases such as muscular dystrophy and lung surfactant deficiencies in newborn.

Proposed Course of Project: The role of membrane phospholipid methylation will be examined in exocytosis, formation of lung surfactant and other membrane events.

Publications:

Axelrod, J.: Physiological and Pharmacological Actions of Adenosylmethionine. In Salvatore, F. Borek, E., Zappia, V. Williams-Ashman, H.G. and Schlenk, F. (Eds.): The Biochemistry of Adenosylmethionine. New York, Columbia University Press, 1977, pp. 539-554.

Hirata, F. and Axelrod, J.: Enzymatic synthesis and rapid translocation of phosphatidylcholine by two methyltransferases in red cell membranes. Proc. Natl. Acad. Sci. USA, 75: 2348-2352, 1978.

Hirata, F., Viveros, O.H., Diliberto, Jr., E.J. and Axelrod, J.: Identification and properties of two methyltransferases in the conversion of phosphatidylethanolamine to phosphatidylcholine. Proc. Natl. Acad. Sci. USA, 75: 1718-1721, 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00428-01 LCS

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)
Benzodiazepine Receptors in Brain

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: S. Paul, Staff Fellow — LCS NIMH

Others: P. Skolnick, Laboratory of Chemistry — NIAMDD
P. Marangos, Clinical Psychobiology Branch — IRP NIMH

COOPERATING UNITS (if any)

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Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	0.0

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A rapid post ictal elevation in the number of [^{3}H] diazepam binding sites has been demonstrated in the rat cortex. After injection of [^{3}H] diazepam into rats, labelling of benzodiazepine receptor in brain has been found. Attempts to isolate an endogenous ligand for the benzodiazepine have shown a number of low molecular weight substances that can compete for [^{3}H] diazepam for receptor sites. The substances have the properties of purine nucleotides. Further studies have established the presence of a heat stable protein (10,000 to 20,000 daltons) isolated from beef cortex that can compete with the high affinity binding of [^{3}H] diazepam. A rapid and sensitive radioreceptor assay for measuring benzodiazepam in plasma has been developed.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: To study the pharmacological and physiological importance of recently described receptors for benzodiazepines in the CNS.

Methods Used: Radioreceptor assays; behavioral techniques; tissue culture.

Major Findings: The effects of experimentally produced drug and electro-shock seizures on high affinity binding of [^{3}H] diazepam to cortical synaptosomes have been studied. A rapid post-ictal elevation in the number of receptors has been demonstrated. This observation may explain the marked efficacy of benzodiazepines in recurrent seizure states such as status epilepticus.

A series of studies on *in vivo* binding of [^{3}H] diazepam in rats has demonstrated that specific labelling of benzodiazepine receptors occurs in various CNS areas following intravenous administration. These results will allow further studies of both receptor occupancy under various pharmacological and physiological states as well as autoradiographic localization of the receptor *in situ*.

Attempts to isolate an endogenous ligand for the benzodiazepine receptor have revealed a number of low molecular weight substances capable of competitively inhibiting binding of [^{3}H] diazepam *in vitro*. These substances do not appear to be peptides but have a number of chromatographic and physical properties characteristic of purine nucleotides. Further studies have established the presence of a heat stable protein with a molecular weight between 10,000 and 20,000 daltons (isolated from bovine cerebral cortex) that is also capable of competitively inhibiting high affinity binding of [^{3}H] diazepam to brain membranes.

A rapid and sensitive radioreceptor assay for measuring benzodiazepines in plasma has been developed. Values derived from this method compare very favorably with the values derived from more cumbersome gas chromatographic methods. This assay will allow routine measurement of the plasma levels of these drugs.

Significance to Biomedical Research: The study of benzodiazepine receptors should further our understanding of the mechanism of action of these anti-anxiety drugs and give some insight into the pathogenesis of seizure states, anxiety, and central control of muscle tone.

Proposed Course of Project: Further studies of benzodiazepine receptors under various physiological and pharmacological states will be carried out using both in vitro and in vivo systems. Purification of putative endogenous ligands to this receptor will also continue.

Publications:

Paul, S.M. and Skolnick, P.: Acute changes in brain benzodiazepine receptors following experimental seizures. Science (in press).

Williamson M.J., Paul, S.M. and Skolnick, P.: Labelling of benzodiazepine receptors in vivo. Nature (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00401-13 LCS

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Formation, Release, Disposition, and Metabolism of Neurotransmitters in Animals

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Irwin J. Kopin	Chief, LCS	LCS NIMH
	Chuang C. Chiueh	Visiting Fellow	LCS NIMH
OTHER:	Gad Gilad	Visiting Fellow	LCS NIMH
	David C. Jimerson	Senior Staff Fellow	LCS NIMH
	Richard Kvetnansky	Visiting Scientist	LCS NIMH
	Richard McCarty	Guest Worker	LCS NIMH
	Nguyen B. Thoa	Pharmacologist	LCS NIMH
	Marta Weinstock	Visiting Scientist	LCS NIMH
	Virginia K. Weise	Chemist	LCS NIMH
	Isamu Yamaguchi	Guest Worker	LCS NIMH
	Anthony P. Zavadil	Guest Worker	LCS NIMH

COOPERATING UNITS (if any)

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Section on Histopharmacology

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NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
6.0	4.8	1.2

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The objectives of this project are to elucidate the biochemical mechanisms controlling the synthesis, storage, release, action and termination of action of neurotransmitters and to determine how genetic factors, environmental stimuli, or drugs influence these control mechanisms. During the last year levels of plasma catecholamines in intact rats have been studied during a variety of stressful conditions in different genetic strains of rats and the relationship of sympathoadrenal responsivity related to behavioral parameters, blood pressure, and heart rate. The effects of chronic treatment with drugs which interfere with sympathetic nerve integrity were studied and the role of peripheral catecholamines in mediating tremor due to oxotremorine examined. The roles of presynaptic receptors in modulating release of norepinephrine from axonal sprouts of organ cultured ganglia were examined.

PROJECT DESCRIPTION: Project Number Z01 MH 00401-13 LCS

Catecholamines in plasma are derived from the adrenal medulla (which secretes mainly epinephrine) and from the sympathetic nerve endings (norepinephrine). Because of their rapid removal from the circulation and the variety of stimuli which evoke their release, the levels of catecholamines in plasma are extremely labile. The development of a technique to obtain from intact, freely moving rats samples of blood and the availability of highly sensitive and specific methods for radioenzymatic assay of epinephrine (EPI), norepinephrine (NE), and dopamine (DA), in plasma has made possible examinations of sympatho-adrenal medullary reactivity. Levels of plasma EPI and NE are increased by even minor changes in the environment. Lifting the top of the cage or moving the cage to another room results in increased plasma levels of EPI and NE. The levels of these amines are strikingly elevated when animals are forcibly immobilized. Plasma levels of EPI reach peak levels (about 40-fold greater than when undisturbed) after about 20 minutes of stress and then decline to about 12-fold greater than basal. Plasma levels of NE are increased by about 6-fold throughout the interval of immobilization. There are also differences among strains in the levels of tyrosine hydroxylase (TH) and dopamine-β-hydroxylase (DBH) activity and of catecholamine content of the adrenal medulla. The least reactive strain had the highest levels of DBH and NE consistent with diminished release of DBH and slower conversion of NE and EPI (although levels of PNMT are not decreased).

There are striking differences among various genetic strains of rats in the plasma catecholamine response evoked by exposure to stress. In a study in which behavioral and plasma catecholamine responses to foot-shock were compared, it was found that there was an excellent correlation between these two parameters in seven different strains of rats. The greatest behavioral activation and the largest increments in plasma catecholamines were found in spontaneously hypertensive (SHR) rats. The normotensive Wistar Kyoto (WKY) strain from which these rats were bred has lower behavioral activation and lesser increases in plasma catecholamines, but these were still high when compared with those found in several other normotensive strains of rats. The strain with the lowest behavioral activation score also had the smallest increment in plasma catecholamines.

The hyper-responsivity to foot-shock of the plasma catecholamine response of SHR rats compared to WKY rats is present at six weeks of age, before development of hypertension, so that the hyper-responsivity is independent of increases in blood pressure (B.P.), but may contribute to its development and maintenance. The B.P. and heart rates (H.R.) could be monitored via the indwelling arterial cannulae and it was found that while transfer of the animals to the shock cage caused greater

increases in B.P. and H.R. in SHR than WKY rats, during the shock intervals the B.P. of SHR decreased. Since H.R.'s were increased, the fall in B.P. was presumably due to vasodilation attending the muscular effort and as a result of the released epinephrine.

A similar pattern emerges when forced immobilization is used as the stressor. During 2 hours of immobilization, circulating levels of NE, EPI, and corticosterone were higher, and remained higher for several hours, in SHR than in WKY rats, but with repeated immobilization there was a reduction in evoked adrenalmedullary secretion and increase in B.P. of SHR rats. One day after the last of a series of six daily intervals of forced immobilization, basal levels of plasma catecholamines were significantly higher, but B.P. was significantly lowered in SHR but not WKY rats. These results suggest that the muscular effort or repeated exposure to high catecholamine levels resulted in reduction of peripheral resistance in SHR rats.

Oxotremorine, a cholinergic (muscarinic-type) agonist, induces tremor in rats by an action in the central nervous system. It has been found to also cause a centrally mediated increase in plasma catecholamines. Since the tremor is partially reversed by removing the adrenal medulla, by treatment with 6-hydroxydopamine (which produces chemical sympathectomy) or by administration of a β-adrenergic blocking agent (although the latter does not prevent the oxotremorine-induced increases in plasma catecholamines, it appears that release of peripheral catecholamines augments the centrally-induced tremor by an action on peripheral β_2-receptors. This may be related to the well known occurrence of tremor attending fear responses in humans.

A new adrenergic blocking agent (EMO 21192) has been found to have a more specific action on the adrenal medulla than does guanethidine, which has its major effect on sympathetic nerve endings. The drug appears to differ from guanethidine since chronic treatment does not produce degeneration of peripheral sympathetic nerves. Because of its selective action on the adrenal medulla a drug of this type may be particular useful in management of severe anxiety states.

Studies on the mechanism of release of catecholamines from isolated tissues e.g., guinea pig vas deferens and axonal sprouts of cultured rat superior cervical ganglia have continued. Stimulation by isoprenaline of β-adrenergic receptors in cultured ganglia enhances release of ^{3}H-NE from axonal sprouts of cultured ganglia. This effect is blocked by (-)propranolol and other β-blocking agents. Thus, presynaptic β-receptors have

a role in the control of release of NE from sympathetic nerve endings. There are also α-adrenergic receptors in superior cervical ganglia but their number, determined by ^{3}H-dihydroergotamine binding, is reduced by decentralization or after 2 days of organ culture. This suggests that the α-receptors are present on cholinergic nerve endings and that there is a feedback mechanism for regulation by catecholamines of release of acetylcholine which is responsible for sympathetic neurone stimulation.

Direct stimulation of preganglionic neurones in the spinal column of pithed rats affords a method for determining the relationship of plasma catecholamine response to elevation of blood pressure and heart rate. The time course of these responses and studies by gas chromatography-mass spectrometry (GC-MS) of metabolites of NE in blood indicate that there is a greater lag in appearance of NE than EPI in the circulation after sympathoadrenal stimulation and that the lag in appearance of metabolites is even greater. This information will be applied to studies in humans in which skin galvonic responses are compared to elevations in plasma catecholamines during procedures which evoke sympathetic neuronal activity.

PUBLICATIONS:

Roizen, M.F., Moss, J., Henry, D., Weise, V., and Kopin, I.J.: Effect of general anesthetics on handling- and decapitation-induced increases in sympathoadrenal discharge. J. Pharmacol. Exp. Ther. 204: 11-18, 1978.

Chiueh, C.C. and Kopin, I.J.: Hyperadrenergic responsivity of hypertensive SHR rat to indirect measurement of blood pressure. Am. J. Physiol., (In press).

Grobecker, H., Roizen, M.F., Jacobowitz, D.M., and Kopin, I.J.: Effect of prolonged treatment with adrenergic neurone blocking drugs on sympathoadrenal reactivity in rats. European J. Pharmacol. 46: 125-133, 1977.

Kopin, I.J.: Noradrenergic Neurotransmission. In Lipton, M.A., DiMascio, A., and Killam, K.F. (Eds.): Psychopharmacology: A Generation of Progress. Raven Press, New York, 1978, pp. 217-219.

Kopin, I.J.: Biochemical assessment of peripheral adrenergic activity. In Paton, D.M. (Ed.): The Release of Catecholamines from Adrenergic Neurons. Pergamon Press, New York, (In press).

Kvetnansky, R., Sun, C.L., Lake, C.R., Thoa, N., Torda, T. and Kopin, I.J.: Effect of handling and forced immobilization on rat plasma levels of epinephrine, norepinephrine and dopamine-β-hydroxylase. Endocrinology, (In press).

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McCarty, R. and Kopin, I.J.: Alterations in plasma catecholamines and behavior during acute stress in spontaneously hypertensive and Wistar Kyoto normotensive rats. Life Sci. 22: 997-1006, 1978.

McCarty, R. and Kopin, I.J.: Sympatho-adrenal medullary activity and behavior during acute stress: A comparison of seven rat strains. Physiol. Behav., (In press).

McCarty, R., Kvetnansky, R., Lake, C.R., Thoa, N.B. and Kopin, I.J.: Sympathoadrenal activity in SHR and WKY rats during recovery from forced immobilization. Physiol. Behav., (In press).

Kvetnansky, R., McCarty, R., Thoa, N.B., Lake, R. and Kopin, I.J.: Sympatho-adrenal responses of spontaneously hypertensive rats to immobilization stress. Am. J. Physiol., (In press).

McCarty, R. and Kopin, I.J.: Pregnancy: Its effects on blood pressure, heart rate and sympatho-adrenal activity in spontaneously hypertensive rats. Proceedings of the Society for Exper. Biol. Med., (In press).

Weinstock, M., Thoa, N.B. and Kopin, I.J.: β-adenoceptors modulate noradrenaline release from axonal sprouts in cultured rat superior cervical ganglia. European J. Pharmacol. 47: 297-302, 1978.

Weinstock, M., Zavadil, A.P., Rosin, A.J., Chiueh, C.C. and Kopin, I.J.: The role of peripheral catecholamines in oxotremorine-tremor in the rat and its antagonism by β-adrenoceptor blocking agents. J. Pharmacol. Exp. Ther., (In press).

Wooten, G.F., Weise, V. and Kopin, I.J.: Norepinephrine synthesis contributes to the accumulation of catecholamines after ligation of rat sciatic nerves. Brain Res. 136: 174-177, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00402-06 LCS

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

CNS Regulation of Autonomic and Endocrine Function

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Irwin J. Kopin	Chief, LCS	LCS NIMH
OTHER:	Chuang C. Chiueh	Visiting Fellow	LCS NIMH
	Richard Kvetnansky	Visiting Scientist	LCS NIMH
	Gertrude Maengwyn-Davies	Guest Worker	LCS NIMH
	Ching-Long Sun	Visiting Fellow	LCS NIMH
	Nguyen B. Thoa	Pharmacologist	LCS NIMH
	Marta Weinstock	Visiting Scientist	LCS NIMH
	Anthony P. Zavadil	Guest Worker	LCS NIMH

COOPERATING UNITS (if any)

Section on Histopharmacology
Section on Pharmacology

LAB/BRANCH
Laboratory of Clinical Science

SECTION
Medicine

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NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.0	3.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to define the regions of brain, the neuronal pathways, and the neurotransmitter systems which control endocrine, metabolic, and autonomic responses to internal stimuli (e.g., blood glucose, 0_2-tension, hormonal state, body temperature, etc.) and to various types of stress (immobilization, foot-shock, hemorrhage, etc.), and the effects of drugs on these systems.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION: Project Number Z01 MH 00402-06 LCS

Using microdissection techniques for removal of specific nuclei in brain, the effects on neurotransmitters and their biosynthetic enzymes are studied. Chemical toxins specific for particular neurones, destruction by electrocoagulation of specific nuclei, or severing neuronal tracts connecting different areas of brain produce alterations in levels of neurotransmitters and enzymes and interfere with physiological responses. Using these techniques, the effects of drugs, hormones, stressors, and genetic factors are being studied.

Oxotremorine is a muscarinic cholinergic drug which produces tremor in rats. The drug induces increases in dopamine (DA) turnover and thereby elevates levels of homovanillic acid (HVA) in the Corpus Striatum (C.S.) and N. Accumbens (N.A.). Both propranolol (a β-receptor blocking agent) and clonidine (an α-receptor stimulator) diminish the oxotremorine-induced increases in HVA in the C.S. but not in the N.A. Haloperidol, which blocks DA receptors also increases levels of HVA in the C.S. and N.A., but its effects are not blocked by either propranolol or clonidine. These results indicated that norepinephrine may mediate the cholinergic stimulation of release of DA in the C.S. but not the N.A.

The turnover rate of NE in the hypothalamus of spontaneously hypertensive (SHR) rats is greater than in normotensive (WKY) rats of the same strain, but turnover of this CA was not increased in the brain stem. This difference was greater in older SHR animals after hypertension is established.

In aggressive strains of mice there appear to be higher levels and increased turnover rates of NE in the hypothalamus, frontal cortex and caudate nucleus than in less aggressive strains. Other areas of brain have higher NE levels but turnover rates are not significantly different.

Epinephrine and phenylethanolamine-N-methyltransferase (PNMT), the epinephrine-forming enzyme, are found in the same small regions of brain. The results suggest that there is an epinephrine-containing neuronal system with cell bodies in the brain stem. The axons from these cells innervate other areas of the brain stem, the locus coeruleus and the hypothalamus. After an interval of forced immobilization of rats, epinephrine levels in the periventricular, ventromedial, and arcuate nuclei are decreased, while in the median eminence, levels of epinephrine are increased, suggesting that the epinephrine-containing cells play a role in the response to stress.

Cocaine, by its excitatory action in the central nervous system, enhances release of epinephrine from the adrenal medulla

into the circulation. This activation may occur at several levels of the CNS, including the spinal cord. Decapitation, presumably as a result of activation of sympathetic preganglionic neurones via injury potentials in the spinal cord, produces striking elevations in plasma catecholamines. The release of catecholamines evoked by decapitation is enhanced by previous activated states of the sympathoadrenal medullary system and depressed by general anesthetics. This may be the result of an altered "set" of sympathetic responsivity in the spinal cord.

The blood brain barrier normally excludes circulating catecholamines from the central nervous system but with osmotic- or hypertension-induced opening of the barrier, catecholamines may enter brain. The physiological consequences of this phenomena are still undetermined.

Using plasma levels of catecholamines to reflect sympathoadrenal medullary secretion and corticosterone levels to reflect release of ACTH from the pituitary, the effects of complete and partial hypothalamic deafferentation on stress-evoked endocrine responses have been examined. Complete hypothalamic deafferentation diminishes markedly the increases in catecholamines and corticosterone found during immobilization or after administration of 2-deoxyglucose (2-DG). Partial deafferentation by severing lateral hypothalamic pathways diminishes the adrenal cortical responses while anterior deafferentation enhances slightly the corticosterone release to immobilization but not to 2-DG. Posterior hypothalamic cuts prevent the elevation of catecholamines by immobilization but do not appear to affect the response to 2-DG. These results suggest that stimulation of the hypothalamus by both immobilization and 2-DG is intiated by neurones outside the hypothalamus and that both anterior and lateral pathways are involved. The discharge of catecholamines appears to involve pathways which pass through the hypothalamus and emerge posteriorly.

Direct stimulation of the hypothalamus evokes release of catecholamines (mainly epinephrine) into the plasma. Direct injection of picrotoxin into the posterior hypothalamus also elicits catecholamine release and this response is prevented by diazepam. The effects of other drugs which alter neurotransmitter release or stimulate receptors are being examined to attempt to ellucidate the neurotransmitters involved in the responses to stress. The responses to posterior hypothalamic stimulation are markedly attenuated by clonidine or α-methyldopa. These drugs are used as antihypertensive agents in patients.

PUBLICATIONS:

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Chiueh, C.C., Sun, C.L., Kopin, I.J., Fredericks, W.R. and Rapoport, S.I.: Entry of [^{3}H] Norepinephrine, [^{125}I] Albumin and Evans blue from blood into brain following unilateral osmotic opening of the blood-brain barrier. Brain Res. 145: 291-301, 1978.

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Tizabi, Y., Thoa, N.B., Maengwyn-Davies, G.D., Kopin, I.J. and Jacobowitz, D.M.: Behavioral correlation of catecholamine concentration and turnover in discrete brain areas of three strains of mice. Brain Res., (In press).

Saavedra, J.M., Kvetnansky, R., and Kopin, I.J.: Adrenaline, noradrenaline and dopamine levels in specific brain stem areas of acutely immobilized rats. Brain Res., (In press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00403-05 LCS

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Control of Catecholamines and DBH in Human Plasma and CSF

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Irwin J. Kopin	Chief, LCS	LCS NIMH
	C. Raymond Lake	Staff Psychiatrist	LCS NIMH
Other:	Virginia K. Weise	Chemist	LCS NIMH
	Michael H. Ebert	Chief, Sect. on Exp. Ther.	LCS NIMH
	Michael D. Coleman	Nephrologist, Holt Krock Med. Clin., Fort Smith, Ark.	
	Dorothy Krieger	Prof. Med., Mt. Sinai School of Med.,	New York
	James H. Wood	Neurologist	SN NIH
	Peter Blombery	Guest Worker	LCS NIMH
	Edna K. Gordon	Chemist	LCS NIMH

COOPERATING UNITS (if any) Section on Experimental Therapeutics, LCS; Holt Krock Medical Clinic, Fort Smith, Arkansas; Dept. of Medicine, Mt. Sinai Hospital, New York, N.Y.; Surgical Neurology Branch (NINCDS), NIH, Bethesda, Maryland

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Laboratory of Clinical Science

SECTION
Medicine

INSTITUTE AND LOCATION
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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.0	2.0	1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Dopamine (DA), norepinephrine (NE), and epinephrine (EPI) and their metabolites and dopamine-β-hydroxylase (DBH) are present in human plasma and cerebrospinal fluid (CSF). Levels of these substances reflect the balance between their release and removal. The increases in levels of NE and EPI with standing, exertion, stress, drugs, administration, etc., are used to examine sympathetic neuronal responsivity. The responses and level of activity of the sympathetic nervous system in cardiovascular disorders (hypertension, hypotension) in alcoholism, a variety of psychiatric disorders, and after treatment with drugs are being examined. The levels of these substances and their metabolites in blood and CSF are examined to attempt to determine the contribution of brain toward the total amine metabolized in the body.

PROJECT DESCRIPTION:

Blood pressure is maintained by changes in sympathetic neuronal activity mediated by the central nervous system which received information from baroreceptors in the aorta and carotid sinus and volume receptors in the right atrium. Plasma levels of norepinephrine (NE) reflect acute changes in sympathetic neuronal activity because the catecholamine is rapidly metabolized and its levels are maintained by continuous secretion into the blood. Dopamine-β-hydroxylase (DBH) in plasma is derived from sympathetic nerves, but its levels are genetically determined and change slowly in any individual because it is slowly metabolized--in a single individual, changes in levels of DBH reflect chronic changes in sympathetic activity or in fluid distribution.

Decreases in plasma volume, as a result of treatment with diuretics, in normal or hypertensive patients, evoke reflex-mediated increases in sympathetic activity reflected by increased levels of plasma NE, but not DBH.

The enhanced sympathetic activity and elevation in plasma levels of NE attending treatment of hypertensive patients with drugs which block actions of catecholamines, produce vasodilation, or diminish vascular volume, can be prevented by administration of drugs, such as clonidine or α-methyl dopa, which activate α-adrenergic receptors in the central nervous system. Fenfluramine, a drug which interacts with serotonergic neurones, also diminishes plasma catecholamines by an unknown mechanism involving the central nervous system.

In our studies, as well as in several other reports, levels of NE in hypertensive patients are similar to those in normal subjects, but the relationship of NE levels in plasma to age found in normal subjects is not apparent in the hypertensive population, presumably due to the presence among the hypertensive subjects of a few young patients with high levels of plasma NE. In other studies hypertensive patients have been found to have higher levels of plasma NE than in normotensive subjects. In these studies the relationship of plasma NE levels to age was not found. Analyses of the results of both groups of studies shows that the difference between the two groups is a result of a difference in the normal subjects. Studies in which hypertensives were found to have higher levels of plasma NE than in normal subjects had values for normal controls which were significantly lower, and had a lower variance, than did the normotensive subjects in studies in which hypertensives and normotensive subjects had similar levels of plasma NE. This difference may be attributed to use of a biased group of older normal subjects. This bias appears to have been introduced by use of medical personnel who were familiar with hospital procedures and failed to have the usual sympathetic response to the procedures found in random normotensive and hypertensive subjects.

Increased levels of NE are evoked by stress, sexual excitation, hypoglycemia, and volume depletion during hemodialysis for chronic renal failure. In patients with Down's syndrome plasma NE levels are normal and respond normally to stress, but levels of DBH in plasma are low, perhaps on a genetically related basis.

Using GC-MS methods for assay of metabolites of catecholamines it appears possible that plasma and urinary metabolites can be used, with appropriate isotopic techniques, for assessing the rate of brain amine metabolism. Pilot studies indicate that nearly half the HVA and between 20 and 40% of urinary MHPG is derived from brain (see also project Z01 MH 00351-04 LCS in Section on Experimental Therapeutics).

NE has been found in human CSF, its levels correlate with those in plasma and are increased when high doses of probenecid are administered.

Treatment of alcoholics with disulfiram causes increases in plasma NE and in blood pressure, perhaps by an action in the central nervous system.

PUBLICATIONS:

Kopin, I.J.: Measuring turnover of neurotransmitters in human brain. In Lipton, M.A., DiMascio, A. and Killam, K.F. (Eds.): Psychopharmacology: A Generation of Progress. New York, Raven Press, 1978, 933-942.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00404-07 LCS

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Growth Characteristics and Immunological Localization of Enzymes in Neurones

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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Other:	Donald E. Schmechel	Medical Officer	LCS	NIMH
	Gad Gilad	Visiting Fellow	LCS	NIMH
	Antonia Liuzzi	Visiting Scientist	LCS	NIMH
	Fredrik G. Foppen	Guest Worker	LCS	NIMH

COOPERATING UNITS (if any)

Clinical Psychobiology Branch, NIMH and Laboratory of Neuropathology and Neuroanatomical Sciences, NIH

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Laboratory of Clinical Science

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Medicine

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.7	1.2	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Immunological methods are being developed in attempts to define the distribution in the nervous system and other tissues of cells (neurones, glia, etc.) which contain specific enzymes associated with particular functions (e.g., production of specific neurotransmitters, hormones, etc.). The ontogeny of the specific proteins and their role in growth, differentiation and regenerative processes are being examined using these techniques.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION:

Nerve growth factor (NGF) or dexamethasone treatment of newborn rats elevate levels of phenylethanolamine N-methyl transferase (PNMT) in sympathetic ganglia. Dexamethasone stimulates increases in epinephrine, but NGF does not, suggesting differences in availability to the enzyme of its substrates. Antibodies to PNMT are being prepared to attempt to determine differences in the location of the enzyme evoked by dexamethasone and NGF.

Antibodies to two different forms of the enzyme, enolase, one of which appears to be specific for neurones (NSE) whereas the other is present in non-neuronal cells and has been called non-neuronal enolase (NNE), have been raised in rabbits. Using these antibodies NSE has been found to replace NNE in immature nerve cells of the cerebellum after the neurones migrate to their final location and begin to mature. NSE has been demonstrated in peptide secreting cells outside the nervous system. This observation suggests a common neuronal origin of these cell types and indicates that they may have similar biochemical processes.

Methods for separation of purified enzymes are being developed to attempt to localize by immunological techniques, neurones with specific characteristics. This will aid in defining neuronal pathways and neurotransmitter systems as well as facilitate studies of development of the nervous system.

Changes in carboxymethylation of proteins in the superior cervical ganglion are being examined to determine the role of the methylating enzyme in maturation and growth of axonal sprouts after injury of axons.

PUBLICATIONS:

Liuzzi, A., Foppen, F.H. and Kopin, I.J.; Stimulation and maintenance by nerve growth factor of phenylethanolamine-N-methyltransferase in superior cervical ganglia of adult rats. Brain Res. 138: 309-315, 1977.

Schmechel, D.E., Marangos, P.J., Zis, A.P., Brightman, M.W. and Goodwin, F.K.: Brain enolases as specific markers of neuronal and glial cells. Science 199: 313-315, 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00378-04 LCS

PERIOD COVERED

October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Immunofluorescence Study of the Uptake of the Antibody to Dopamine-beta-Hydroxylase in the Rat Brain

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PIS:	David M. Jacobowitz	Chief, Sect. on Histopharm.	LCS	NIMH
	Michael A. Silver	Guest Worker	LCS	NIMH

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NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.1	1.1	

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

We previously reported the specific in vivo uptake and retrograde transport of antibody to dopamine-β-hydroxylase (ADβH) by noradrenergic (NA) neurons in the rat brain. The objective of the present study was to further explore this preliminary observation: (1) To examine the process by which ADβH is taken up into NA neurons, and (2) to utilize this unique uptake and retrograde flow phenomenon to elucidate the circuitry and origin of NA innervations to specific brain regions. Pharmacological destruction of adrenergic neurons by 6-hydroxydopamine blocked the uptake of ADβH; further evidence that this immunoglobulin is taken up exclusively into dopamine-β-hydroxylase (DβH)-containing neurons. Pharmacological manipulations with adrenergic agents suggest that the ADβH uptake process is related to the norepinephrine (NE) release mechanism. Minute volumes of ADβH were stereotaxically injected into 41 sites within the spinal cord, brainstem, diencephalon and telencephalon of anesthetized rats. Utilizing the specific ADβH uptake and retrograde flow characteristic of NE neurons, it was possible to elucidate the specific NE circuitry to each of these areas and the associated pathways. This neuroanatomical technique is presently the most sensitive technique for the detection and elucidation of CNS NE circuitry.

Project Description:

Objectives: (1) To utilize the characteristics of specific uptake and retrograde transport of antibody to dopamine-β-hydroxylase by noradrenergic neurons as a neuroanatomical tool to elucidate the specific circuitry of NA projections to various brain structures. (2) To study the process by which antibody to dopamine-β-hydroxylase is selectively taken up by noradrenergic neurons in the rat brain in vivo.

Methods Employed: Immunohistochemical localization of ADβH by an indirect immunofluorescence technique utilizing a fluorescein-labelled antiserum to the primary antibody. The antibody to DβH was injected intraventricularly (5 µl) or intracerebrally (0.1 - 0.4 µl) into the rat brain. The rats were killed between 24 and 48 hrs.

Major Findings: Pharmacological lesion studies utilizing intraventricular (ivt) and intracerebral injections of 6-hydroxydopamine verified our earlier finding that CNS DβH-containing neurons selectively take up ADβH in vivo. Acceleration of NE release by NE neurons following treatment with phentolamine (1 mg/kg i.p.) or clonidine (0.1 mg/kg i.p.) enhanced the intensity of ADβH staining by the immunofluorescence technique within parikarya of the locus coeruleus after the ivt administration of the immunoglobulin. Inhibition of locus coeruleus firing activity with clonidine (1.0 mg/kg i.p.) decreased the intensity of ADβH staining. These results suggest that ADβH uptake is related to the release of NE from synaptic vesicles which contain DβH.

Retrograde flow mapping studies utilizing stereotaxic injections of ADβH elucidated the relative contributions of each NE cell group (A1-A6 and subcoeruleus) to NE innervation of the hypothalamic nuclei, basal ganglia, hippocampal formation, thalamus, cortical areas, limbic areas, spinal cord and brainstem structures. This study also demonstrated that the locus coeruleus (A6 cell group) receives NE projections from each of the other NE cell groups. This represents the first finding of a CNS NE neuron projecting to a NE neuron in another cell group.

Significance: The observation that intracerebrally administered ADβH is specifically taken up by CNS NE neurons and transported intraneuronally via a retrograde flow process to the associated cell bodies provides a number of significant findings. (1) This technique offers a rapid, simple procedure for the visualization of central NE neurons. (2) The observed retrograde transport of ADβH suggests the presence of an endogenous DβH retrograde transport process. The significance of this finding is as yet unknown. (3) As a practical neuroanatomical tool, this technique offers the most sensitive method for the elucidation of central noradrenergic circuitry. The results obtained with this technique suggest a possible topographical as well as functional organization within the NE innervation patterns of the brain. (4) The finding that ADβH is related to the synaptic release of NE may be of significance to the study of synaptic events and transport process and may serve as a model for the uptake and retrograde transport of other retrograde flow labels used as neuroanatomical tools such as horseradish peroxidase. (5) The finding that NE cell bodies of the locus coeruleus receive NE innervations from other NE cell groups represents an additional regulatory process for NE neurons. (6) The observation that a CNS

neuron is capable of specifically taking up an immunoglobulin molecule and transporting it via retrograde flow processes to the cell body may be highly significant to the study of neuropathological, neurovirological and neuroimmunological processes.

Proposed Course of Project: This project, having completed its initial goals has reached completion. The final experiments are currently in the process of finishing. This last study will determine the effect of intraneuronal ADβH on the activity of these neurons as measured by the levels of norepinephrine in cell body and terminal areas of the rat brain.

Publication:

Silver, M.A. and Jacobowitz, D.M.: In vivo uptake and retrograde flow of antibody to dopamine-β-hydroxylase: A new specific technique for the visualization of central noradrenergic neurons. Brain Res., in press, 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER ZO1 MH 00381-04 LCS

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)
Central Noradrenergic Control Over Pituitary Oxytocin and Vasopressin

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PIS:	David M. Jacobowitz	Chief, Sect. on Histopharm.	LCS	NIMH
	William R. Crowley	Guest Worker	LCS	NIMH
	Thomas L. O'Donohue	Guest Worker	LCS	NIMH
*				

COOPERATING UNITS (if any)

* Jack M. George
Ohio State Univ., School of Medicine, Dept. of Med.

LAB/BRANCH
Laboratory of Clinical Science

SECTION
Section on Histopharmacology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Md. 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	0.3	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Rat pituitary levels of oxytocin were elevated by a single injection of estrogen. This treatment did not affect pituitary levels of vasopressin. However, a second injection of estrogen or an injection of progesterone to estrogen-pretreated rats raised vasopressin levels. Norepinephrine levels and turnover in the paraventricular nucleus were reduced by hormone treatments that elevated pituitary vasopressin, while norepinephrine in the supraoptic nucleus did not change. Transection of the ascending ventral noradrenergic bundle reduced norepinephrine levels in the hypothalamic nuclei and also reduced pituitary vasopressin, but markedly elevated pituitary oxytocin.

PHS-6040
(Rev. 10-76)

Project description:

Objectives: To investigate the effects of ovarian hormones on pituitary levels of oxytocin and vasopressin and to study the possible mediation of these effects by noradrenergic systems.

Methods Employed: (1) Radioimmunoassays for oxytocin and vasopressin. (2) Microdissection of hypothalamic nuclei. (3) Enzymatic-isotopic assay for norepinephrine. (4) Stereotaxic neurosurgery of the rat.

Major Findings: Rat pituitary levels of oxytocin were elevated by a single injection of estrogen. This treatment did not affect pituitary levels of vasopressin. However, a second injection of estrogen or an injection of progesterone to estrogen-pretreated rats raised vasopressin levels. Norepinephrine levels and turnover in the paraventricular nucleus were reduced by hormone treatments that elevated pituitary vasopressin, while norepinephrine in the supraoptic nucleus did not change. Transection of the ascending ventral noradrenergic bundle reduced norepinephrine levels in the hypothalamic nuclei and also reduced pituitary vasopressin, but markedly elevated pituitary oxytocin.

Significance to Biomedical Research and the Program of the Institute: These findings support a role for posterior pituitary hormones in reproductive processes such as fertilization. Furthermore, they indicate an important role for noradrenergic systems in the control of posterior pituitary hormones with the possibility that a noradrenergic system is a substrate for ovarian-neurohypophyseal interaction.

Proposed Course of Project: Follow-up studies include further analysis of noradrenergic control by lesioning noradrenergic neurons with the neurotoxin 6-hydroxydopamine and determination of ovarian hormone effects on oxytocin and vasopressin in brain nuclei.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER ZO1 MH 00382-04 LCS

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Biochemical Mapping of Monoaminergic Neurons of the Rat Brain

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PIS:	David M. Jacobowitz	Chief, Sect. on Histopharm.	LCS	NIMH
	Thomas L. O'Donohue	Guest Worker	LCS	NIMH
	William R. Crowley	Guest Worker	LCS	NIMH

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Clinical Science

SECTION
Histopharmacology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.3	0.2	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This study maps, by biochemical means, the ascending projections of the noradrenergic ventral bundle of the rat brain by the use of a microdissection technique capable of removing specific brain nuclei combined with a sensitive isotopic assay for norepinephrine and dopamine. Transection of the noradrenergic ventral bundle resulted in a reduction of the norepinephrine concentration in the nucleus accumbens, lateral septum, median eminence, central gray, nucleus interstitialis striae terminalis and the ventromedial, dorsomedial, medial amygdaloid, paraventricular, arcuate, supraoptic, medial preoptic, lateral preoptic and paraventricular nuclei. A decrease in the nucleus accumbens dopamine concentration and turnover and an increase in the dopamine content and turnover of the nucleus tractus diagonalis suggest specific noradrenergic-dopaminergic interactions.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: To map, biochemically, the ascending projections of the ventral noradrenergic bundle and investigate possible interactions with dopaminergic systems.

Methods Employed: (1) Bilateral and unilateral transection of the noradrenergic ventral bundle were made stereotaxically. Animals were sacrificed after 12 days and brains dissected by the micropunch technique (Palkovits). (2) Regions were assayed for norepinephrine and dopamine by a radioenzymatic assay.

Major Findings: Areas which showed a significant decrease in norepinephrine concentration after bilateral ventral bundle transection were: the nucleus accumbens, lateral septum, median eminence, central gray, nucleus interstitialis striae terminalis and the ventromedial, dorsomedial, medial amygdaloid, periventricular, arcuate, supraoptic, medial preoptic, lateral preoptic and paraventricular nuclei. Dopamine concentrations and turnover decreased in the nucleus accumbens and increased in the tractus diagonalis. These changes in dopamine systems after bilateral disruption of a noradrenergic system suggests a noradrenergic-dopaminergic interaction. Suprisingly, unilateral lesions caused increases in norepinephrine regions such as the nucleus interstitialis striae terminalis, the lateral preoptic, the paraventricular, and the anterior hypothalamic nuclei. These changes may be due to processes of recovery of function such as neuronal sprouting.

Significance to Biomedical Research and the Program of the Institute: This study provides a detailed description and quantitation of the noradrenergic projections from the ventral bundle to discrete regions of the rat brain. Knowledge of the anatomy of noradrenergic systems is a prerequisite for studying behavioral, physiological and neurochemical interactions of norepinephrine in the central nervous system. A neurochemical interaction between norepinephrine and dopamine in limbic and striatal areas such as the nucleus tractus diagonalis and the nucleus accumbens may provide valuable information to the understanding of the effects of transmitters in various states of behavior.

Proposed Course of Project: Biochemical mapping of neurotransmitter systems and their neurochemical interactions in the brain will continue.

Publication:

Speciale, S.G., Crowley, W.R., O'Donohue, T.L., and Jacobowitz, D.M.: Forebrain catecholamine projections of the A5 cell group. Brain Res., in press, 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00383-04 LCS

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

The Effects of Stress on Catecholamine Concentration in Discrete Regions of the Rat Brain

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PIS:	David M. Jacobowitz	Chief, Sect. on Histopharm.	LCS	NIMH
	John A. Moyer *	Guest Worker	LCS	NIMH

COOPERATING UNITS (if any)
* Lorraine R. Herrenkohl
Temple University, Phila. Penna.

LAB/BRANCH
Laboratory of Clinical Science

SECTION
Section on Histopharmacology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0		

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Project completed with publication of results.

PHS-6040
(Rev. 10-76)

Publication:

Moyer, J. A., Herrenkohl, L. R. and Jacobowitz, D. M.: Stress during pregnancy: Effect on catecholamines in discrete brain regions of offspring as adults. Brain Res. 144: 170-178.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER ZO1 MH 00386-03 LCS

PERIOD COVERED

October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Studies of the Brains of Genetically Obese Rats

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PIS:	David M. Jacobowitz	Chief, Sect. on Histopharm.	LCS	NIMH
	Nguyen B. Thoa	Pharmacologist	LCS	NIMH
*				

COOPERATING UNITS (if any)

Section on Medicine, Laboratory of Clinical Science, NIMH
* Judith A. F. Cruce, John Hopkins Univ., Baltimore, Md.

LAB/BRANCH

Laboratory of Clinical Science

SECTION

Section on Histopharmacology

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Md. 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Levels of norepinephrine and dopamine were measured in eight discrete regions of the hypothalamus in three groups of male rats: genetically obese, non-obese and castrated non-obese. DA levels showed no significant differences among the groups in any of the regions. NE levels in the paraventricular nucleus were significantly lower in the obese and castrated animals than in the normal animals. In the median eminence, NE levels were significantly decreased for the castrated group. None of the other regions sampled showed significant differences in NE levels.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: In a recent study of obese Zucker rats (fafa) it was observed that the norepinephrine concentration was different in two regions (paraventricular nucleus and median eminence) compared to that of non-obese controls (FaFa and Fa-). The subjects of that study were female rats of approximately four months of age. The present experiment is an attempt to extend these original observations by using younger male rats. Since female Zucker rats are abnormal in reproductive function the abnormalities in catecholamine levels might be related to changes in gonadal hormone levels. Therefore a group of castrated animals of the same age was included for comparison.

Methods Employed: (1) Microdissection technique for removal of discrete nuclei. (2) Enzymatic-isotopic microassay for catecholamines. (3) Surgical castration.

Major Findings: (1) There was a significant decrease in the norepinephrine concentration of the paraventricular nucleus of both the fatty and castrated rats compared with controls. (2) In the median eminence norepinephrine levels were significantly decreased for the castrated group.

Significance to Biomedical Research and the Program of the Institute: The fact that the NE levels in the paraventricular nucleus decreased in both castrated and fatty rats is interesting since fatties show decreased testicular size which could result in a reduced amount of circulating androgens and would, in effect, mimic castration. Furthermore, the paraventricular nucleus is a target tissue for androgen (and estrogen) uptake as demonstrated by autoradiographic studies. That the paraventricular nucleus may be critical for the production of follicle stimulating hormone-releasing factor (FSH-RF) is suggested by the observation that lesions of the paraventricular nucleus cause a decrease in the concentration of FSH-RF. The above observations suggest that the reproductive impairment of fatty rats may be linked to gonadal hormones, the paraventricular nucleus and gonadotropic release from the pituitary gland.

Proposed Course of Project: Terminated with a manuscript submitted for publication.

Publications:

Cruce, J.A.F., Thoa, N.B. and Jacobowitz, D.M.: Catecholamines in discrete areas of the hypothalamus of obese and castrated male rats. Physiol. Behav. 8: 287-289, 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00388-02 LCS

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)
Neurochemical and Histochemical Studies of Cholinergic Pathways of the Brain

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PIS:	David M. Jacobowitz	Chief, Sect. on Histopharm.	LCS	NIMH
	Donald B. Hoover	Guest Worker	LCS	NIMH
	Zehava Gottesfeld	Guest Worker	LCS	NIMH

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Clinical Science

SECTION
Section on Histopharmacology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Md. 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.4	1.4	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

1. The dorsal tegmental acetylcholinesterase (AChE)-containing pathway was lesioned in the midbrain reticular formation. The lesions involved AChE-positive perikarya in the n. cuneiformis. A 4 day survival period was allowed before examination of the brain for histochemical changes in AChE and biochemical changes in choline acetyltransferase (CAT). A decrease in AChE staining and CAT activity was observed in the ipsilateral anterior thalamic nuclei, n. reticularis thalami, n. medialis thalami, n. parafascicularis, the pretectal nuclei, the lateral geniculate and the superior colliculus. CAT, unlike AChE, is a specific marker for cholinergic neurons. In the areas with decreased staining for AChE, CAT activity was also decreased to 40-60% of control values obtained from sham rats. This study provides evidence that a major cholinergic pathway emanates from the region of the n. cuneiformis.

2. The nucleus of the tractus diagonalis was found to contain a major cholinergic pathway that projects via the stria medullaris, habenula, and fasciculus retroflexus to provide 50% of the cholinergic innervation to the interpeduncular nucleus.

Project Description:

Objectives: (1) To provide evidence for or against the suggestion that a large cholinergic projection ascends in the brain from the reticular formation. (2) To examine the utility of acetylcholinesterase (AChE) as a marker for cholinergic neurons when used in combination with biochemical measurements of choline acetyltransferase (CAT). (3) To investigate a possible cholinergic pathway from the nucleus tractus diagonalis to the interpeduncular nucleus.

Methods Employed: (1) Unilateral radiofrequency lesions were placed in the area of AChE-positive cell bodies in the nucleus cuneiformis or nucleus tractus diagonalis. (2) After various time periods rats were decapitated and their brains were removed, frozen, and sectioned in a cryostat. (3) Thin sections were stained for AChE (Koelle), 300 µm sections were employed for microdissection. (4) Brain regions were removed from the 300 µm section by the microdissection technique of Palkovits. (5) CAT activity was assayed in this tissue by the radiometric method of Fonnum.

Major Findings: (1) High CAT activity is found in a reticular formation area (nucleus cuneiformis) with AChE-positive cell bodies. (2) Placement of a lesion in this nucleus and the surrounding region results in marked ipsilateral decreases in AChE staining. (3) Parallel drops occur for CAT measured in tissue adjacent to that examined histochemically. (4) Apparent projection areas include the anterior thalamic nuclei, the pretectal and deep pretectal nuclei, the lateral geniculate, several other thalamic nuclei, and the lower layers of the superior colliculus. (5) Lesions placed in the nucleus of the tractus diagonalis resulted in a loss of 50% of CAT activity in the interpeduncular nucleus.

Significance to Biomedical Research and the Program of the Institute: Evidence has been provided for the existence of an extensive cholinergic dorsal tegmental pathway. This is a key starting point for studies aimed at determining functions of acetylcholine in the brain.

The cholinergic innervation to the interpeduncular nucleus emanates, almost equally, from the habenula and the nucleus of the tractus diagonalis. Experimental manipulation of this cholinergic tract may help to reveal the physiological significance of the interpeduncular nucleus.

Proposed Course of Project: The next phase of this study will be to determine whether nerve terminals in the proposed projection areas arrive from the AChE-positive cell body region of the reticular formation. This will be accomplished by following the retrograde transport of horseradish peroxidase.

Studies will be carried out on the interaction of cholinergic neurons with other neurotransmitter-specific neurons (substance P, serotonergic, GABAergic, noradrenergic) in the interpeduncular nucleus following lesion of the nucleus of the tractus diagonalis.

Publications:

Hoover, D.B., Muth, E.A. and Jacobowitz, D.M.: A mapping of the distribution of acetylcholine, choline acetyltransferase, and acetylcholinesterase in discrete areas of rat brain. Brain Res., in press, 1978.

Hoover, D.B., Muth, E.A. and Jacobowitz, D.M.: A method for sectioning microwave-fixed brain prior to microdissection and acetylcholine analysis. Neuroscience Letters 5: 247-251, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00389-02 LCS

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Effects of Ovarian Hormones on Plasma Gonadotropins and on Catecholamines in Brain Nuclei

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PIS:	David M. Jacobowitz	Chief, Sect. on Histopharm.	LCS	NIMH
	William R. Crowley	Guest Worker	LCS	NIMH
	Thomas L. O'Donohue	Guest Worker	LCS	NIMH
	Helena Wachslicht	Guest Worker	ERRB	NICHD

COOPERATING UNITS (if any)
Developmental Endocrinology Section, Endocrinology & Reprod. Research Branch, NICHD

LAB/BRANCH
Laboratory of Clinical Science

SECTION
Section on Histopharmacology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Md. 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.9	0.5	0.4

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Treatment of ovariectomized female rats with estrogen lowered plasma follicle stimulating hormone and luteinizing hormone 54 hr later and reduced the turnover of norepinephrine in the interstitial nucleus of the stria terminalis and central gray and of dopamine in the nucleus tractus diagonalis. Treatment of estrogen-primed females with progesterone at 48 hr elevated plasma gonadotropins at 54 hr and increased norepinephrine turnover in the interstitial nucleus of the stria terminalis, central gray and arcuate nucleus and increased dopamine turnover in the nucleus tractus diagonalis and interstitial nucleus of the stria terminalis. Estrogen plus progesterone decreased norepinephrine turnover in the ventromedial, periventricular and paraventricular nuclei.

PHS-6040
(Rev. 10-76)

Project description:

Objectives: To determine the effects of ovarian hormones on catecholamine levels and turnover in individual brain nuclei of ovariectomized female rats.

Methods Employed: (1) The microdissection technique to remove individual brain nuclei. (2) The enzymatic-isotopic microassay for catecholamines. (3) Radioimmunoassays for follicle stimulating hormone and luteinizing hormone.

Major findings: Treatment of ovariectomized female rats with estrogen lowered plasma follicle stimulating hormone and luteinizing hormone 54 hr later and reduced the turnover of norepinephrine in the interstitial nucleus of the stria terminalis and central gray and of dopamine in the nucleus tractus diagonalis. Treatment of estrogen-primed females with progesterone at 48 hr elevated plasma gonadotropins at 54 hr and increased norepinephrine turnover in the interstitial nucleus of the stria terminalis, central gray and arcuate nucleus and increased dopamine turnover in the nucleus tractus diagonalis and interstitial nucleus of the stria terminalis. Estrogen plus progesterone decreased norepinephrine turnover in the ventromedial, periventricular and paraventricular nuclei.

Significance to Biomedical Research and the Program of the Institute: The present studies demonstrate effects of ovarian hormones on the activity of central catecholamine systems in specific regions of the brain. These effects can be correlated with alterations in secretion of pituitary hormones. These results are relevant to neural control of gonadotropin secretion, ovulation and mating behavior.

Proposed Course of Project: Further studies are in progress concerning the effects of ovarian hormones on serotonergic, GABAergic and cholinergic systems in discrete areas of the brain.

Publications:

Crowley, W.R., O'Donohue, T.L. and Jacobowitz, D.M.: Changes in catecholamine content in discrete brain nuclei during the estrous cycle of the rat. Brain Res., 147, p. 315-326, 1978.

Crowley, W.R., O'Donohue, T.L., Wachslicht, H. and Jacobowitz, D.M.: Effects of estrogen and progesterone on plasma gonadotropins and on catecholamine levels and turnover in discrete brain regions of ovariectomized rats. Brain Res., in press, 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00391-02 LCS

PERIOD COVERED October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Neurochemical and Anatomical Studies of GABAergic Neurons

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PIS:	David M. Jacobowitz	Chief, Sect. on Histopharm.	LCS	NIMH
	Zehava Gottesfeld	Guest Worker	LCS	NIMH

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Clinical Science

SECTION
Histopharmacology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.8	0.8	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Evidence is presented in support of the suggestion that the stria medullaris (SM) contains GABAergic afferents to the lateral habenula. It was found that the lateral (LHb) and medial (MHb) habenular nuclei differentially accumulate ^{3}H-GABA and ^{3}H-glutamate by a 4:1 ratio each, respectively. After SM lesion the uptake of GABA decreases in the LHb (62%) and glutamate uptake decreases in the MHb (50%).

Experiments designed to chemically characterize the habenulo-raphe pathway have not supported prior electrophysiological studies which suggest that this is a GABAergic pathway.

PHS-6040
(Rev. 10-76)

Project description:

Objectives: To map the distribution of GABAergic neurons in discrete brain regions.

Methods Employed: (1) Bilateral lesions of the stria medullaris or habenula were placed in rat brains. (2) Dissection of lateral and medial habenula from Vibratome-cut sections. (3) GAD activity (Fonnum method) and GABA uptake by synaptosomal preparations. (4) Brain microdissection method (Palkovits).

Major Findings: (1) The uptake of GABA was higher in the lateral habenula compared to the medial habenula. (2) As a result of SM lesions the capacity of the lateral habenula to take up GABA was lost by 41% and 62% after survival of 4 and 35 days, respectively. This supports the suggestion that GABAergic fibers within the stria medullaris project to the lateral habenula. (3) No change in GAD activity was observed in the dorsal raphe nucleus following lesion of the habenula.

Significance to Biomedical Research and the Program of the Institute: The habenular nuclei have been implicated in a number of behavioral and physiological manifestations, and have been considered as a relay station between fore- and midbrain limbic structures. The identity of neurotransmitters projecting to and emanating from this region to other target areas in the brain may provide firstly, basic information on the interaction amongst neurotransmitters in the CNS and secondly, their involvement in brain function and defined behavioral patterns controlled by discrete brain regions.

Proposed Course of Project: Studies on the interaction of GABAergic with other neurotransmitter-specific neurons in discrete brain regions are in progress.

Publications:

Gottesfeld, Z. and Jacobowitz, D.M.: Neurochemical and Anatomical Studies of GABAergic Neurons. In Garattini, S., Pujol, J.F. and Samanin, R. (Eds.): Interactions Between Putative Neurotransmitters in the Brain. New York, Raven Press, 1978, pp. 109-126

Gottesfeld, Z., Massari, V.J., Muth, E.A., and Jacobowitz, D.M.: Stria medullaris: A possible pathway containing GABAergic afferents to the lateral habenula. Brain Res. 130: 184-189, 1977.

Gottesfeld, Z., Hoover. D.B., Muth, E.A. and Jacobowitz, D.M.: Lack of biochemical evidence for a direct habenulo-raphe GABAergic pathway. Brain Res., 141: 353-356, 1978.

Gottesfeld, Z. and Jacobowitz, D.M.: Further evidence for GABAergic afferents to the lateral habenula. Brain Res., in press, 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00392-01 LCS

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Effects of Cu(II)-DOPA-ATP Chelates on Brain Dopamine and Metals

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PIS:	David M. Jacobowitz	Chief, Sect. on Histopharm.	LCS	NIMH
	William R. Crowley	Guest Worker	LCS	NIMH
	Thomas L. O'Donohue	Guest Worker	LCS	NIMH
	Robert W. Colburn	Chemist	LCS	NIMH
*				

COOPERATING UNITS (if any)

* Krishnaswamy S. Rajan
IIT Research Institute

LAB/BRANCH
Laboratory of Clinical Science

SECTION
Section on Histopharmacology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Md. 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.4	0.3	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Administration of low doses of Cu(II)-DOPA-ATP to normal rats markedly elevated dopamine levels in the caudate nucleus, nucleus accumbens and olfactory tubercle when compared with equimolar doses of L-DOPA. In reserpine plus tranylcypromine-pretreated rats in which dopamine levels were depleted, Cu(II)-DOPA-ATP restored dopamine levels to normal while DOPA had no effect in the dosage used. Both DOPA and DOPA-chelate decreased copper levels in the brain nuclei.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: To determine the efficacy of Cu(II)-DOPA-ATP chelate to elevate brain dopamine levels.

Methods Employed: (1) The microdissection technique to remove individual brain nuclei. (2) The enzymatic-isotopic microassay for catecholamines.

Major Findings: Administration of low doses of Cu(II)-DOPA-ATP to normal rats markedly elevated dopamine levels in the caudate nucleus, nucleus accumbens and olfactory tubercle when compared with equimolar doses of L-DOPA. In reserpine plus tranylcypromine-pretreated rats in which dopamine levels were depleted, Cu(II)-DOPA-ATP restored dopamine levels to normal while DOPA had no effect in the dosage used. Both DOPA and DOPA-chelate decreased copper levels in the brain nuclei.

Significance to Biomedical Research and the Program of the Institute: These results suggest that metal chelates of DOPA enter the brain more readily than DOPA and thus may become useful in treatment of neurological diseases involving dopamine (e.g. Parkinson's Disease).

Proposed Course of Project: Further studies will analyze dose-response and time-response relationships and may examine the neurochemical effect of DOPA chelate on neurologically impaired primates.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00393-01 LCS

PERIOD COVERED October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Effects of Suckling on Regional Brain Catecholamines and Turnover in Post-Partum Rats

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PIS:	David M. Jacobowitz	Chief, Sect. on Histopharm.	LCS	NIMH
	Thomas L. O'Donohue *	Guest Worker	LCS	NIMH

COOPERATING UNITS (if any) * John A. Moyer, Temple University
Lorraine Herrenkohl, Temple University
Richard Gala, Wayne State University

LAB/BRANCH Laboratory of Clinical Science

SECTION Histopharmacology

INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.3	0.1	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This study investigates the effects of suckling on catecholamine content and turnover of discrete brain regions of mother rats. Both suckling and treatment with α-methyl-para-tyrosine the catecholamine synthesis blocker, resulted in a marked increase in serum prolactin levels. Suckling caused a decrease in norepinephrine content and turnover in the anterior hypothalamic nucleus. The ventromedial nucleus responded to suckling with increases in norepinephrine and dopamine.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: To investigate the effects of suckling on catecholamine content and turnover in discrete regions of the brains of mother rats.

Methods Employed: Norepinephrine and dopamine were assayed by a radioenzymatic technique (Henry) in discrete regions (Palkovits) of suckling rat brains. Turnover was assessed by determination of the relative depletion of norepinephrine or dopamine after inhibition of synthesis with alpha-methyl-para-tyrosine.

Major Findings: Both suckling and treatment with alpha-methyl-para-tyrosine resulted in a marked increase in serum prolactin levels. Suckling caused a decrease in norepinephrine content and turnover in the anterior hypothalamic nucleus. The ventromedial nucleus responded with an increased dopamine content and an increased norepinephrine content. These changes may be associated with the behavioral or neuroendocrine events associated with suckling.

Significance to Biomedical Research and the Program of the Institute: This study investigates interactions between the endocrine and behavioral changes associated with suckling and norepinephrine and dopamine in discrete regions of the rat brain. The changes in catecholamine content or turnover in the anterior hypothalamus and the ventromedial nuclei of suckling rats may indicate the involvement of these neurotransmitter systems in neuroendocrine regulation of prolactin or oxytocin release.

Proposed Course of Project: Further studies on the relationships between suckling and other neurotransmitter systems are in progress.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00394-01 LCS

PERIOD COVERED: October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

The Role of Central Cholinergic Mechanisms in the Feedback Actions of Gonadal Steroids

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PIS:	David M. Jacobowitz	Chief, Sect. on Histopharm.	LCS	NIMH
	Eric A. Muth	Biologist	LCS	NIMH
	William R. Crowley	Guest Worker	LCS	NIMH

COOPERATING UNITS (if any)

LAB/BRANCH: Laboratory of Clinical Science

SECTION: Histopharmacology

INSTITUTE AND LOCATION: NIMH, ADAMHA, NIH, Bethesda, Md. 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.4	0.4	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This study seeks to delineate the role of central cholinergic neurons in the regulation of gonadal steroid hormone levels in rats. Changes in the choline acetyltransferase (ChAT) activity of discrete brain areas in response to the manipulation of circulating levels of gonadal hormones have been used to identify those areas which may participate in the feedback actions of gonadal steroids. In adult male rats, castration elevated ChAT activity in the medial preoptic nucleus and posterior medial amygdala. Testosterone treatment lowered ChAT activity in the nucleus tractus diagonalis. In female rats ovariectomized as adults, estradiol treatment followed 48 hr later by progesterone reduced ChAT activity in the caudal nucleus tractus diagonalis and in the periventricular nucleus and increased ChAT activity in the supraoptic nucleus. Progesterone alone also reduced ChAT activity in the caudal nucleus tractus diagonalis.

These brain areas are among those known to be targets for testicular and ovarian hormones, and may represent sites where circulating gonadal steroids initiate neuroendocrine feedback actions through cholinergic mediators.

PHS-6040
(Rev. 10-76)

Project Description:

Objective: To describe the role of central cholinergic systems in the regulation of gonadal function via the neuroendocrine axis.

Methods Employed: (1) Male rats were castrated or sham-operated at 60 days of age. One group of castrated rats received testosterone propionate (TP) and another group sesame oil vehicle daily for 14 days. Sham controls also received oil. (2) Female rats, ovariectomized four weeks previously, received either benzoate or sesame oil followed 48 hr later by either progesterone (P), or oil, and were killed at 54 hr. (3) Twenty-five discrete brain areas were removed by the microdissection technique of Palkovits. (4) Choline acetyltransferase (ChAT) activity was measured by the radioisotopic assay of Fonnum.

Major Findings: (1) In male rats, castration significantly elevated ChAT activity in the medial preoptic nucleus and in the posterior medial amygdala, while TP lowered ChAT activity to the sham level in the latter area. The TP treatment also significantly decreased ChAT activity in the nucleus tractus diagonalis. (2) In female rats, EB+P reduced ChAT activity in the caudal nucleus tractus diagonalis and in the periventricular nucleus. EB+P increased ChAT activity in the supraoptic nucleus. P alone also reduced ChAT activity in the caudal nucleus tractus diagonalis.

Significance to Biomedical Research and the Program of the Institute: Pharmacological manipulation of cholinergic neurons has provided evidence that central cholinergic mechanisms may be involved in the regulation of gonadotropin secretion by the anterior pituitary and vasopressin/oxytocin secretion by the posterior pituitary. The brain areas discussed above are among those known to be gonadal hormone targets as well as among those known to be involved in neuroendocrine secretion in the rat. Cholinergic neurons may provide a link, either directly or in concert with other neurotransmitter systems, between the steroid hormone receptors in these areas and gonadotropin releasing hormone or oxytocin/vasopressin neurosecretion in the hypothalamus.

The EB+P regime used in this study stimulates an LH "surge" reminiscent of that seen just prior to ovulation. The cholinergic responses in the periventricular nucleus and caudal nucleus tractus diagonalis may be part of the sequence of events which signals ovulation; alternatively, they may be involved in the sexual behavioral responses associated with estrus. Acetylcholine is thought to stimulate vasopressin neurosecretion in the supraoptic nucleus, and the present acceleration of cholinergic activity in this area may reflect a hormonally-mediated effect on vasopressin and/or oxytocin secretion over the estrous cycle.

It is suggested that central cholinergic neurons are directly or indirectly involved in the neuroendocrine regulation of gonadal function. When fully characterized, the operation of these cholinergic systems may help explain the reciprocal interrelationships among psychological state, the limbic system, gonadal and gonadotrophic hormones, and sexual behavior.

Proposed Course of Project: Acetylcholine levels will be measured in discrete rat brain areas after gonadal hormone manipulation as a further

indication of cholinergic involvement in gonadotropin regulation. The radioimmunoassay of plasma levels of FSH and LH is currently underway, and will facilitate quantifying the pituitary neurosecretory response to cholinergic manipulation of discrete brain areas.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00351-04 LCS

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Clinical Pharmacology of the Central Nervous System

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. H. Ebert	Chief, Sec. on Exp. Therapeutics	LCS NIMH
OTHER:	I. J. Kopin	Chief, Lab. of Clinical Science	LCS NIMH
	M. A. Elchisak	Staff Fellow	LCS NIMH
	C. R. Lake	Staff Psychiatrist	LCS NIMH
	E. Gordon	Head, Unit on Clinical Biochemistry	LCS NIMH
	R. Polinsky	Staff Physician	LCS NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Clinical Science

SECTION
Section on Experimental Therapeutics

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.5	2.5	1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this study is to develop techniques for studying central nervous system monoamine metabolism in man, and to carry out pharmacokinetic studies of psychoactive drugs. Studies of the kinetics and clearance of deuterated homovanillic acid (HVA) from the circulation were conducted in rhesus monkeys and patients. HVA distributes in the body according to a two compartment model, and is cleared with a β half life of approximately 45 minutes. Infusion of deuterated homovanillic acid, in combination with probenecid infusion, may provide a method for estimation of the rate of production of HVA by the central nervous system in man. CSF probenecid levels measured during the oral probenecid test are significantly correlated with elevations in HVA and 5HIAA throughout the range of probenecid concentrations encountered. Studies on CSF norepinephrine (NE) levels suggest that it is a more satisfactory indicator of brain NE metabolism than NE metabolite levels in CSF.

Project Description:

Objectives: 1. To develop techniques for studying kinetic parameters of central nervous system monoamine metabolism in man. 2. To carry out pharmacokinetic studies of psychoactive drugs. 3. To utilize these techniques to study patients with a variety of psychiatric, neuropsychiatric, and psychosomatic disorders.

Methods Employed:

1. Biochemical methods: Flourometric, gas chromatographic, and mass spectrometric methods are used for assay of endogenous catecholamine metabolites in tissues and body fluids. Mass spectrometric methods are used for assay of deuterium labelled catecholamine metabolites administered exogenously or synthesized endogenously. Gas chromatographic and radioimmunoassay techniques are used for assay of drugs in body fluids (probenecid and d-amphetamine).

2. Physiological methods: As the project progresses, an increasing number of metabolic experiments will be carried out in patients. However, as new procedures are being developed, appropriate experiments will be carried out in rat brain tissue and in primates using serial sampling of blood, cerebrospinal fluid, and urine as necessary. To facilitate these preclinical *in vivo* experiments on brain metabolism, a small primate metabolic laboratory has been developed with the capacity to maintain chronic collection of lateral ventricle or lumbar cerebrospinal fluid from chaired rhesus monkeys for days or weeks at a time.

Major Findings:

Studies involving the use of deuterium-labelled homovanillic acid (HVA), the major metabolite of dopamine, to document *in vivo* changes in dopamine metabolism have been extended this year in primates. Rhesus monkeys were infused with deuterated HVA and serial samples of CSF, blood, and urine were obtained for analysis of endogenous and deuterated HVA by gas chromatography-mass spectrometry. Less than 1% of HVA in CSF is derived from blood. The kidney does not contribute a significant amount of HVA from renal metabolism of dopamine to the urine. HVA distributes in the body according to a two-compartment model, with a β half life of approximately 45 minutes, an elimination rate constant of .032 min^{-1}, a volume of distribution of .88 liter/kg, and a renal clearance of 50 ml/min. The results, in combination with ongoing clinical experiments, suggest that infusion of deuterated HVA, in combination with probenecid infusion, will be a practical method of estimation of the rate of production of HVA by brain (Drs. Elchisak, Polinsky, Ebert, and Kopin).

Studies of the relationship of CSF probenecid levels to the change in acid metabolites of serotonin (5HIAA) and dopamine (HVA) in the 100 mg/kg probenecid test have continued (Drs. Ebert, Cowdry, Goodwin, Lake). A significant positive correlation exists throughout the range of CSF probenecid levels encountered (10-45 µg/ml). The contribution of differences in probenecid absorption between patients undergoing probenecid tests should be accounted for by an analysis of covariance. Lithium treated subjects have significantly

lower CSF probenecid levels than controls. Probenecid treatment (100 mg/kg increases CSF norepinephrine levels.

Drs. Gordon, Ebert, Kopin, and Blombery have further developed a technique for estimation of central nervous system norepinephrine (NE) synthesis rate by labelling peripheral stores of NE with ^{14}C-dopamine (^{14}C-DA) and measuring the specific activities of VMA and MHPG in the urine. The method assumes that negligible amounts of VMA originate in the central nervous system, and that the observed difference in specific activity of VMA and MHPG in the urine reflects the amount of unlabelled MHPG originating from metabolism of NE in the central nervous system. The analytical chemistry in this experiment has been improved, using high pressure liquid chromatography to separate labelled urinary metabolites, and using gas chromatography-mass spectrometry to determine the endogenous amounts of MHPG and VMA. Data is being collected on normal subjects and several disease groups with suspected or known disorders of NE metabolism.

Studies have continued on the physiological significance of CSF NE levels (Drs. Lake, Ebert, Gordon, Perlow, Ziegler). A study in which NE, MHPG, and VMA were measured in CSF chronically collected from the lateral ventricle of a rhesus monkey demonstrated that a distinct diurnal rhythm is present in NE levels, but that an apparently random fluctuation exists in the levels of the two major metabolites of NE. There is a clear dose dependent increase in CSF NE after 0.25-1.0 mg/Kg of d and l amphetamine. Essentially no NE appears in lateral ventricle CSF after an infusion of radiolabelled NE into the circulation of the rhesus monkey. These studies strengthen our impression that CSF NE may be a better indicator of brain NE metabolism than CSF levels of NE metabolites.

Studies of the pharmacokinetics of amphetamine in hyperactive children, and the relationship of blood levels of amphetamine to clinical response have continued. A study to determine the bioavailability and pharmacokinetic parameters of d-amphetamine and methylphenidate began this year. A complete discussion of these projects is found in Projects Z01 MH 00100-03 AP and Z01 MH 00040-02 AP of the Biological Psychiatry Branch.

Significance to Biomedical Research:

Assessing the rate of amine metabolite formation in animals and in patients provides information on the rate of amine utilization. The development of more quantitative methods for determining central nervous amine utilization in man is essential for testing hypotheses regarding the role of amines in psychiatric, medical, and neurological disorders and assessing the effects of drugs on the amines in the brain.

Proposed Course:

The techniques described above are now being applied to studies in patients. When appropriate, some of the above techniques will also be studied next year in normal volunteers in order to generate a comparison group to patients with various disorders of neurotransmission.

Publications:

1. Ebert, M.H. and Perlow, M.J.: Utility of cerebrospinal fluid measurements in studies of brain monoamines. In Usdin, E., Weiner, N. and Youdim, M.B.H. (Eds.): Structure and Function of Monoamine Enzymes. New York, Marcel Dekker, Inc., 1977, pp. 963-984.

2. Marynick, S.P., Smith, G.B., Ebert, M.H., and Loriaux, D.L.: Studies on the transfer of steriod hormones across the blood-cerebrospinal fluid barrier in the rhesus monkey. II. Endocrinology 101: 232-237, 1977.

3. Lake C.R., Wood, J.H., Ziegler, M.G., Ebert, M.H., and Kopin, I.J.: Probenecid-induced norepinephrine elevations in plasma and CSF. Arch. Gen. Psychiatry 35: 237-240, 1978.

4. Perlow, M., Ebert, M.H., Gordon, E.K., Ziegler, M.G., Lake, C.R., and Chase, T.N.: The circadian variation of catecholamine metabolism in the subhuman primate. Brain Res. 139: 101-113, 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00352-03 LCS

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Pharmacological and Psychometric Studies of Neuropsychiatric Syndromes

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. H. Ebert	Chief, Sec. on Exp. Therapeutics	LCS NIMH
OTHER:	E. D. Caine	Medical Officer	LCS NIMH
	H. Gross	Staff Psychiatrist	LCS NIMH
	R. Polinsky	Staff Physician	LCS NIMH
	H. Weingartner	Acting Chief	LPP NIMH
	C. Ludlow	Speech Pathologist	CDP NINCDS

COOPERATING UNITS (if any)
Communicative Disorders Program, NINCDS
Laboratory of Psychology, NIMH
Laboratory of General and Comparative Biochemistry, NIMH

LAB/BRANCH
Laboratory of Clinical Science

SECTION
Section on Experimental Therapeutics

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	2.0	0.5

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords) Several neuropsychiatric syndromes including Huntington's chorea, Gilles de la Tourette's syndrome are being studied in terms of memory function, neuroendocrine function, and possible pharmacological treatment. The pathophysiology and neuropharmacology of anorexia nervosa and orthostatic hypotension is also under investigation. Acute pharmacological studies of memory in Huntington's chorea and normals indicate that the transient memory disorder induced by scopolomine in normal controls has similar features to the dementia of Huntington's chorea. A double blind study of clozapine, a non-neuroleptic antipsychotic, in Gilles de la Tourette's syndrome was negative, as was a double blind, crossover trial of desmethyl-imipramine and chlorimipramine. The latter trial raises doubts concerning the involvement of serotonin in the pathophysiology of Tourette's syndrome. A study of the neuropharmacology of orthostatic hypotension is underway, which includes assessment of CNS and sympathetic nervous system norepinephrine (NE) metabolism, and dose-response to pressor infusions. A double blind trial of lithium carbonate in anorexia nervosa suggests that it is efficacious. Plasma NE and urinary execretion of catecholamine metabolites are significantly reduced during the acute phase of anorexia nervosa, but return to normal following recovery.

Project Description:

Objectives: The purpose of this study is to investigate several neuropsychiatric syndromes from the perspective of memory and intellectual function, neuroendocrine function, neurotransmitter metabolism, and possible pharmacological treatment. Currently under study are several dementias with known neuropathology, particularly Huntington's dementia and Altzheimer's disease. Other diseases under intensive study are Gilles de la Tourette's syndrome, anorexia nervosa, and orthostatic hypotension.

Methods Employed: Methods for studying information processing in neuropsychiatric disorders include learning tasks that permit the assessment of stimulus and modality specific information in processing (1) short term memory; (2) long term memory; (3) retrieval processing; (4) encoding; (5) the interrelationships of these processes and (6) factors that might influence decay and interference of memory in these systems. Procedures are used to test free recall, serial learning, cued recall, and recognition memory. These methods have previously been validated and studied in a number of clinical and psychopharmacological studies. The procedures to measure short term memory functions include immediate recall of random high and low imagery words, consonant trigrams, taxonomically organized words, digits, and forms generated from mutually exclusive binary dimensions. Long term memory functions are tested by using equivalent sets of stimuli, with varying periods of delay. Rehearsal is prevented through the insertion of appropriate, noninterfering tasks. Differences between free recall and various cued recall conditions is examined. Encoding strategies associated with normal versus disturbed brain states is measured using sorting tasks having redundant and random word stimuli.

One strategy for identifying neurotransmitter systems that may play a role in the pathogenesis of psychiatric or neurological symptoms is to study the clinical effects of a series of drugs having relatively specific stimulating or blocking effects on a particular neurotransmitter. This approach has been profitably applied to research on affective disorders. We are pursuing a pilot investigation of this nature in patients with dementias of various etiologies, focusing on the adrenergic and cholinergic systems. A similar study of drugs that potentiates noradrenergic or serotonergic neurotransmission has been completed in Gilles de la Tourette's syndrome.

Methods used in neuroendocrine protocols are established techniques of studying plasma prolactin and growth hormone responses to the acute administration of drugs known to affect dopaminergic neurotransmission. From these endocrine responses one can infer the functional state of dopaminergic transmission in the tuberoinfundibular system. Methods used in Stage II drug trials are established methods for the evaluation of psychotropic drugs including double blind design, appropriate rating scale placebo controls. Methods used in studies of neurotransmitter metabolism are those methods outlined in Project ZO1 MH 00351-02 LCS.

Major Findings: Pharmacological studies of memory function in Huntington's chorea and in normal subjects have continued this year (Drs. Caine, Weingartner and Ebert). Parameters of memory processing and retrieval in

Huntington's chorea are not significantly changed by acute administration of physostigmine, haloperidol, or amphetamine. In normal subjects, scopolamine causes a marked, transient memory disorder which is characterized by 1) failure to distinguish between high and low imagery words, 2) failure to benefit from semantic processing of words (as opposed to acoustic processing), 3) inability to perform selective reminding tasks, 4) delayed serial learning, and 5) difficulty remembering simple geometric designs. This memory impairment appears to be similar to that of Huntington's chorea, and perhaps of Alzheimer's disease. After amphetamine, normal subjects show enhanced recall, particularly on selective reminding.

A biochemical genetic protocol in Huntington's chorea has been initiated with Dr. Carl Merril and Dr. John Leavitt (FDA). Two diminsional polyacrilamide gel electrophoresis is a new technique that can resolve several thousand proteins in a biological sample. The technique is being applied to serum, cerebrospinal fluid, and cultured skin fibroblasts in an attempt to identify and characterize abnormal proteins in Huntington's chorea and in relatives at risk for the disorder.

The research program on Gilles de la Tourette's syndrome has now evaluated nearly 60 patients. This past year approximately 15 were admitted for pharmacologic trials. A double blind study of clozapine, a non-neuroleptic antipsychotic, showed that although clozapine was pharmacologically active (causing significant side effects) it did not have a therapeutic action. Chlorimipramine and desipramine were also studied as possible treatments for Tourette's syndrome in a double blind, crossover design. Neither drug was found to be efficacious. This result raises doubts regarding serotonergic and noradrenergic mechanisms playing a major role in the pathophysiology of the syndrome (Drs. Caine, Polinsky, Ebert).

A study was completed on hypoxanthine phosphoribosyl transferase (HGPRT) in Gilles de la Tourette's syndrome, an enzyme of purine metabolism that has been reported to be abnormal in this disease (Drs. Merril, Leavitt, Ebert and Caine). We found no abnormality in the activity or the stability of the enzyme in Tourette's patients in comparison with control subjects.

The Tourette's program has continued to collect family history data. At this point, over 2000 patients and relatives have been sampled, with 32 out of the first 50 families having a positive history for motor tics, vocal tics, or Tourette's syndrome. This study is particularly significant as the sample obtained is substantially different from previously reported populations (Miss Nee; Drs. Caine, Ebert; Dr. Eldridge, NINCDS). Linguistic studies have continued in collaboration with Dr. Christy Ludlow with an aim of discerning whether the apparent language impairment found in many patients is transient and disappears with maturation, or whether it causes a permanent disability.

An inpatient evaluation of various types of orthostatic hypotension has begun this year. The protocol has three phases: diagnostic evaluation; dose response to infusion of norepinephrine (NE), tyramine, and angiotensin; and clinical trials. The aim of the project is to classify orthostatic hypotension

into etiological categories, and to improve or expand the neuropharmacological treatment of these patients. The current evaluation includes autonomic testing (e.g., Valsalva maneuver, hyperventilation, cold pressor and sweat test, pupillary responses), plasma renin, urinary aldosterone excretion rate after salt restriction, ^{51}Cr-labelling of red cells for plasma volume and RBC mass, plasma NE, CSF NE and amine metabolites, turnover studies for central and peripheral norepinephrine and dopamine pools. Analysis of plasma NE reveals four response patterns: normal - normal supine NE level with 100% increase on standing; Shy-Drager syndrome - normal supine NE with a small increase on standing; peripheral - low supine NE with minimal increase on standing; and orthostatic tachycardia - normal supine NE with two to five fold increase on standing (Drs. Polinsky, Lake, Ebert, Kopin).

A clinical research program in primary anorexia nervosa (PAN), has been underway this year with 3-4 patients in treatment in a behavior modification program (Drs. Gross, Ebert, Lake, Kopin). A double blind trial of lithium carbonate in comparison with placebo is nearing completion. Six patients on lithium increased their percent ideal body weight 13.3 $\pm$ 1.4% versus 10.9 $\pm$ 1.7% for 8 patients on placebo. A preliminary trial of delta-9-tetrahydrocannabinol (delta-9-THC) in a double blind crossover design with diazepam is in progress. No behavioral toxicity of delta-9-THC has been encountered in this patient group. Plasma levels of NE and urinary excretion of HVA and MHPG were studied before and after significant clinical improvement in anorexia nervosa patients and compared with normal females. During the active phase of the disease, blood pressure and pulse were lower than those of normal subjects, and increased with weight gain. When acutely ill, plasma levels of NE, and urinary excretion of MHPG and HVA were significantly lower than controls. After weight gain, these parameters increased to normal levels. The results suggest that abnormalities in catecholamine metabolism in PAN are secondary to changes in body weight (patients had adequate nutritional intake when the initial studies were performed). A study of insulin receptor sites with Drs. Wachslicht and Roth (NIAID) indicate that patients with PAN have an increase in insulin binding sites on both RBC and WBC, a finding opposite to that observed in obese patients. Further studies of cardiac function in PAN have demonstrated that left ventricular, left atrial, and aortic root dimensions on echocardiogram are below the range of normal adult values at baseline, but increase to normal after nutritional rehabilitation.

Significance to Biomedical Research:

Despite a European tradition of neuropsychological research throughout this century, it has only been in the past 15-20 years that interest in cognitive functioning has increased significantly in this country, as seen for example in the works of Talland, Goodglass, or Sperry. The biological mechanisms which underlie memory processing remain unclear. In recent years, interest has increased in the biochemical aspects of learning and memory. Neuropharmacological aspects of memory function have also become a focus of interest. Most of the published studies in this area are in animals. However, there is a beginning literature of clinical neuropharmacological investigations of memory, including work on stages of memory storage pursued recently in our

program by Drs. Weingartner and Murphy. We hope to extend knowledge about pharmacological and biochemical aspects of memory function by studying several diseases in which disordered memory is a primary symptom, and in which there is existing knowledge about anatomical and biochemical pathology.

Gilles de la Tourette' syndrome is a neuropsychiatric syndrome whose pathophysiology is poorly understood. The symptomatology appears to be responsive to drugs which block dopamine receptors. By conducting new drug trials, and gathering data on cognitive and linguistic function, monoamine metabolism, and neuroendocrine function in this illness, we hope to expand knowledge about its pathophysiology and its possible relationship to other psychiatric illnesses such as schizophrenia. Anorexia nervosa is another neuropsychiatric syndrome in which there is little published information concerning pharmacological treatment and neurotransmitter metabolism. It is clearly established in the animal literature that monoamine systems in the hypothalamus play an important role in the normal regulation of appetite.

Proposed Course:

The course of this project is related to the development of 2 West as a central nervous system clinical pharmacology service. New diseases may come under study as indicated by available laboratory techniques in the LCS. In the next year we intend to further pursue pharmacological studies of memory function in Huntington's chorea, Altzheimer's disease, and normal controls. We plan to apply two dimensional polyacrilamide gel electrophoresis of plasma and CSF proteins to Huntington's chorea. In Gilles de la Tourette's syndrome we intend to pursue neurochemical studies such as those indicated in Project Z01 MH 00351-04, and further drug trials. In anorexia nervosa we plan to conclude a drug trial of Δ-9-tetrahydrocannabinol; and extend studies of cardiovascular, gastrointestinal and autonomic nervous system function during and after the acute phase of the illness. In orthostatic hypotension we plan to conclude the first phase of the neurochemical and pharmacological differentiation of these patients.

Publications:

1. Caine, E.D., Hunt, R.D., Weingartner, H., and Ebert, M.H.: Huntington's dementia. Arch. Gen. Psychiatry 35: 377-384, 1978.

2. Caine, E.D., Ebert, M.H., and Weingartner, H.: An outline for the analysis of dementia. Neurology 27 (11): 1087-1092, 1977.

3. Weingartner, H., Caine, E.D., and Ebert, M.H.: Imagery, encoding and the retrieval of information from memory: Some specific encoding-retrieval changes in Huntington's disease. J. Abnorm. Psychol., In press.

4. Weingartner, H., Caine, E.D., and Ebert, M.H.: Experimental psychological approaches to a human memory disorder: information storage and retrieval processes in Huntington's disease. Neuropsychologia, In press.

5. Caine, E.D., Kartzinel, R. Ebert, M.H., and Carter, A.C.: Neuroendocrine function in Huntington's disease: dopaminergic regulation of prolactin release. Life Sci. 22: 911-918, 1978.

6. Brown, W.A., Corriveau, D.P., and Ebert, M.H.: Acute psychologic and neuroendocrine effects of dextroamphetamine and methylphenidate. Psychopharmacology, In press.

7. Caine, E.D., Margolin, D.I., Brown, G.L., and Ebert, M.H.: Gilles de la Tourette's syndrome, tardive dyskinesia, and psychosis in an adolescent. Am. J. Psychiatry 135 (2): 241-242, 1978.

8. Merril, C.R., Leavitt, J., Van Keuren, M.L., Ebert, M.H., and Caine, E.D.: Hypoxanthine guanine phosphoribosyltransferase (HGPRT) in Gilles de la tourette syndrome. Neurology, In press.

9. Gottdiener, J.S., Gross, H.A., Henry, W.L., Borer, J.S., and Ebert, M.H.: Effects of self-induced starvation on cardiac size and function in anorexia nervosa. Cardiology, In press.

10. Gross, H.A., Lake, C.R., Ebert, M.H., Ziegler, M.G., and Kopin, I.J.: Catecholamine metabolism in primary anorexia nervosa. In press. N. Engl. J. Med.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00271-09 LCS

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)
Measurement of Brain Contribution to Norepinephrine Metabolites in Human Urine

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Irwin J. Kopin	Chief, LCS	LCS	NIMH
Other:	Edna K. Gordon	Chemist	LCS	NIMH
	Michael H. Ebert	Chief, Sect. on Exp. Ther.	LCS	NIMH
	Robert L. Sherman	Chemist	LCS	NIMH
	Peter Blombery	Guest Worker	LCS	NIMH
	Jerry A. Oliver	Chemist	LCS	NIMH
	Sanford P. Markey	Pharmacologist	LCS	NIMH

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Clinical Science

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.5	2.0	1.5

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Gas liquid chromatography-mass spectrometry (GLC-MS), ion exchange chromatography, high pressure liquid chromatography (HPLC) and other separation procedures are used to isolate and measure the specific activities of the norepinephrine metabolites, 3-methoxy, 4-hydroxyphenylglycol (MHPG) and vanillyl mandelic acid (VMA) in the urine of controls and patients with mental and neurologic disorders.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION:

In humans, norepinephrine (NE) in the sympathetic nervous system and the adrenal medulla is converted to vanillyl mandelic acid (VMA) and 3-methoxy, 4-hydroxyphenylglycol (MHPG). Small amounts 1-5% of the MHPG occur in the free form and the rest is conjugated as glucuronide or sulfate in roughly equal quantities. VMA formed is about 1 to 2 times that of total MHPG. In brain, MHPG is the main metabolite of NE, with only very small amounts of VMA being produced. Previous work of this and other laboratories suggest that MHPG, in free form, is removed from brain directly to the blood.

When ^{14}C dopamine is infused peripherally it does not enter brain. About 25% of it is converted to ^{14}CNE and its metabolites. Since almost all VMA is formed outside the brain its specific activity (ratio of radioactive to non-radioactive VMA) will be characteristic of peripheral metabolism. The specific activity of MHPG will represent peripheral plus brain metabolism. The differences in specific activity of total MHPG from that of VMA will be a measure of the amount of MHPG from brain. The specific activities of the free, glucuronide or sulfate of MHPG may be an indication of the form in which MHPG is transported from brain.

Radioactive and deuterated standards of VMA and of the three forms of MHPG have been prepared. Separation procedures using ion exchange and high pressure liquid chromatography (HPLC) have been worked out. Final assay is done by gas liquid chromatography-mass spectrometry (GLC-MS) and scintillation counting.

Some assays of controls and patients treated with ^{14}C dopamine have been completed. The specific activities of MHPG glucuronide and sulfate are similar suggesting that MHPG is removed from brain as the free compound and conjugated in the periphery. The few control urines assayed so far suggest that the strategy is a useful one. Urines of patients and controls are being collected and analyzed.

PUBLICATIONS:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00272-07 LCS

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Metabolism, Distribution and Biochemical Effects of Psychoactive Drugs

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Sanford P. Markey	Pharmacologist	LCS	NIMH
Other:	Robert W. Colburn	Chemist	LCS	NIMH
	Anthony P. Zavadil	Guest Worker	LCS	NIMH
	Alfred J. Lewy	Staff Psychiatrist	CPB	NIMH
	Richard L. Hawks	Guest Worker	LCS	NIMH
	G. John Shaw	Visiting Fellow	CH	NHLBI
	Irwin J. Kopin	Chief, LCS	LCS	NIMH
	Michael H. Ebert	Chief, Sect. on Exp. Ther.	LCS	NIMH

COOPERATING UNITS (if any)

Sections on Medicine and Experimental Therapeutics, LCS; Clinical Psychobiology Branch, NIMH; Laboratory of Chemistry, NHLBI

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2.0	1.5	0.5

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☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Studies on the biochemical effects of drugs in animals and man using mass spectral methodology are being pursued in order to relate pharmacological action to molecular events and clinical efficacy. Work in progress includes the synthesis of stable isotope labeled compounds for kinetic studies in man of neurotransmitters (catecholamine derivatives) and the effects of various drugs upon the synthesis and release of catecholamines. Drug manipulation of melatonin levels is being measured to indicate beta-adrenergic receptor activity. Tricyclic antidepressants (imipramine, desipramine) and their hydroxylated metabolites are being quantitated from patients by mass spectral measurements in order to establish therapeutic and predictive indices.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION:

Synthesis techniques suitable for the preparation of gram quantities of stable isotope labeled compounds have been developed. Deuterated diazomethane of 99.1 mole % D_2 purity can be prepared from diazomethane and D_2O using phase transfer catalysis.

Monomethylated catecholamine metabolites have been prepared from catechol and iodomethane-D_3 on the gram scale in purity suitable for clinical studies in humans. The deuteromethyl analogues of homovanillic acid, 3-methoxy-4-hydroxy phenyl glycol and vanillylmandelic acid were synthesized. Future synthetic efforts will be aimed at the production of oxygen-18 labeled catechol to be used as a building block for catecholamine synthesis.

Studies on the metabolism and distribution of bromocryptine in experimental animals have been concluded--no evidence was obtained that the drug requires conversion to an active metabolite in order to exert its action.

Imipramine and desipramine levels have been determined in an effort to establish therapeutic and predictive indices for these commonly used tricyclic antidepressants in collaboration with the Clinical Psychobiology Branch (Z01 MH 00446-08).

Pharmacokinetic studies of cocaine in humans are in progress. Analysis by the GC-MS method for cocaine in human blood samples obtained from a collaborator at NY Medical School will be continued in FY'78 to include at least 12 subjects in total.

A project to test THC as a possible therapeutic agent in anorexia nervosa is underway. Oral THC was administered to approximately five subjects during FY'77 and blood samples were taken at various time points and analyzed by GC-MS at Battelle Laboratories in Ohio. As yet sufficient data have not been collected to correlate blood levels with any clinical effects. This project will continue in FY'78 and should be completed during that year.

Another area of research involved a collaboration with endocrinologists at the NICHD. This study involved the in vitro analysis of specific binding characteristics of marihuana preparations with estrogen receptor preparation. The rat ovary preparation was used and a number of cannabinoids and marihuana extracts were tested. It was concluded that no specific interaction occurred with THC or any single cannabinoid

tested. Marihuana extract itself did react and the source of this interaction was traced to flavanoids in the plant. Two of these endogenous flavanoids were obtained and shown to bind to specific estrogen receptors. These data refute two literature reports that THC binds specifically to estrogen receptors.

Levels of melatonin in human plasma are being quantitated by negative ion chemical ionization mass spectrometry (Z01 MH 00274-04 LCS). Control of the synthesis and release of melatonin from the pineal gland is mediated by a beta-adrenergic receptor. Propranolol, isoproteranol, and various psychoactive drugs will be tested for their effects on this receptor in humans (collaborative with project number Z01 MH 00450-03 CPB).

PUBLICATIONS:

Markey, S.P. and Shaw, G.J.: Preparation of highly enriched diazomethane-d_2. J. Org. Chem. 1978 (in press).

Markey, S.P. and Lewy, A.J.: Quantitative analysis of melatonin in human plasma by negative chemical ionization mass spectrometry. Proc. Ninth Materials Research Symposium, National Bureau of Standards, Gaithersburg, 1978 (in press).

Potter, W.Z., Zavadil, A.P., and Goodwin, F.K.: Prediction of steady-state plasma concentration of imipramine. Psychopharmacology, 1978 (in press).

Rapoport, J.L., Mikkelsen, E.J., and Zavadil, A.P.: Plasma tricyclic level and clinical response in enuresis. Psychopharmacology, 1978 (in press).

Muscettola, G., Goodwin, F.K., Potter, W.Z., Claeys, M.M., and Markey, S.P.: Imipramine and desipramine in plasma and spinal fluid: Relationship to clinical response and serotonin metabolism. Arch. Gen. Psychiatry 35: 621-625, 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00273-09 LCS

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Studies on Synaptosomes

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Robert W. Colburn	Chemist	LCS NIMH
OTHER:	Nguyen B. Thoa	Pharmacologist	LCS NIMH
	Irwin J. Kopin	Chief, LCS	LCS NIMH
	Iain C. Campbell	Visiting Fellow	CN NIMH

COOPERATING UNITS (if any)

Clinical Neuropharmacology Branch, NIMH

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Synaptosomes prepared from various regions of rat brain are used as models of nerve endings to study mechanisms of uptake and release of neurotransmitters in the central nervous system. The distribution of neurotransmitters within synaptosomes are determined by hypotonic shock on millipore filters following neurotransmitter uptake or uptake plus drug-induced release.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION:

Synaptosomes prepared from various regions of rat brain are used as models of nerve endings to study mechanisms of uptake and release of neurotransmitters in the central nervous system. When neurotransmitter uptake is followed over time with hypotonic shock treatment on millipore filters there is a preferential uptake in the shock resistant fraction during early (1-2 min.) time periods. If the accumulated transmitter is released by depolarizing K+ levels there is a calcium-dependent release from the shock resistant fraction and a calcium-independent release from the shock susceptible fraction.

Studies with I.C. Campbell show that monoamine oxidase in synaptosomes is inhibited by clorgyline a type A inhibitor but not by deprenyl a type B inhibitor.

PROPOSED COURSE: Project has been discontinued.

PUBLICATIONS:

Rajan, K.S., Wiehl, R.D., Riesen, W.H., Colburn, R.W. and Davis, J.M.: Effect of metal chelating agents on the storage of norepinephrine in vitro by cerebral synaptic vesicles. Biochem. Pharmacol. 26: 1703-1708, 1977.

Campbell, I.C., Colburn, R., Walker, M.N., Lovenberg, W. and Murphy, D.L.: Observations on the effects of a chronic phenelzine regimen on the metabolism of 5-hydroxytryptamine and norepinephrine in the rat brain. In Deniker, P., Radouco-Thomas, C. and Villeneuve, A. (Eds.): Proceedings of the 10th Congress of the Collegium Internationale Neuropsychopharmacologicum (CINP), Pergamon Press, Oxford, 1977, pp. 629-640.

Rajan, K.S., Colburn, R.W. and Davis, J.M.: Distribution of metal ions in the subcellular fractions of several rat brain areas. Life Sci., 18: 423-432, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00274-04 LCS

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)
Methods of Ionization in Mass Spectrometry

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Sanford P. Markey	Pharmacologist	LCS NIMH
OTHER:	Alfred J. Lewy	Staff Psychiatrist	CPB NIMH
	Robert W. Colburn	Chemist	LCS NIMH
	Henry W. Rosenstock	Institute Scientist	NBS
	Arthur R. Schultz, Jr.	Computer Scientist	CSL DCRT

COOPERATING UNITS (if any)
Clinical Psychobiology Branch, NIMH; Section on Technical Development, NIMH; Computer Systems Laboratory, DCRT, Chemical Thermodynamics Division, National Bureau of Standards

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Negative chemical ionization mass spectrometry has been employed for its enhanced ionization efficiency for the detection and quantitation of electron capturing derivatives of volatile molecules. Melatonin has been quantitated in human plasma at the part per trillion level (picogram/ml) using an improved ion source which contains collinating magnets to stabilize high sensitivity measurements and a discharge plasma as an alternate electron source. N-acetylserotonin and serotonin have also been measured with enhanced sensitivity.

A collaborative NIH-NBS task force has been initiated to study ion source characteristics and determine sensitivity limits by various ionization methods.

A selected ion recording system operating under computer control has been completed in a collaborative effort with CSL/DCRT. Hardware and software are being evaluated on an LKB 9000.

PROJECT DESCRIPTION:

Negative ion chemical ionization mass spectrometry has been tested and improved in a manner which permits the quantification of melatonin in human plasma at the part per trillion level (clinical studies reported in Project Number Z01 MH 00450-03 CPB). Previous mass spectral quantitation in this and other laboratories had been limited to the part per billion level due to decreased ionization efficiency relative to electron capture negative chemical ionization and the relative non-specificity of ionization which resulted in high chemical background signals. Further improvement of ion source operating characteristics has been observed with the installation of electron beam collinating magnets in the negative ion source.

In order to focus the instrumentation design interests of organic mass spectroscopists at NIH, a collaborative study program has been initiated with NBS. The long-term objective of the group is to obtain better ion sources for analytical mass spectrometry. In spite of the femtogram (10^{-15}g) sensitivity realized with the melation study, most ion sources yield only one ion for every million neutral molecules entering the ion source.

In collaboration with this Laboratory, Mr. Luther Barden with A. R. Schultz of CSL/DCRT have designed and implemented a PDP 11/10 computer system to automate ion focusing and data acquisition from a LKB 9000 magnetic defection mass spectrometer. The system allows the user to create and use his own data manipulation routines in a repetitive manner, amenable to multiple quantitative assays. The system is now undergoing testing and final revision.

PUBLICATIONS:

Markey, S.P., Lewy, A.J. and Colburn, R.W.: Quantitative analysis of compounds from biological extracts using negative chemical ionization mass spectrometry. Proc. 26th Ann. Conf. on Mass Spectrometry and Allied Topics, St. Louis, 1978, (In press).

Markey, S.P., Lewy, A.J. and Colburn, R.W.: Studies in biogenic amine metabolism by mass spectrometry. Proc. Int. Symposium on Quantitative Mass Spectrometry in the Life Sciences, Gent, Belgium, 1978 (In press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00275-01 LCS

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Release and Turnover of Catecholamine Metabolites in Human Subjects

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Peter A. Blombery	Guest Worker	LCS	NIMH
Other:	Irwin J. Kopin	Chief, LCS	LCS	NIMH
	Edna K. Gordon	Chemist	LCS	NIMH
	Michael H. Ebert	Chief, Sect. on Exp. Ther.	LCS	NIMH
	Sanford P. Markey	Pharmacologist	LCS	NIMH
	Jerry A. Oliver	Chemist	LCS	NIMH

COOPERATING UNITS (if any)

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NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

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2.5	1.5	1.0

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Methods have been developed for the assay of the catecholamine metabolites, 3-methoxy-4-hydroxy phenylglycol, dihydroxyphenylglycol and dihydroxymandelic acid in plasma by gas chromatography-mass spectrometry. In the monkey, intravenously administered deuterated 3-methoxy-4-hydroxy phenylglycol is cleared rapidly from the circulation and secreted as sulfate and glucuronide conjugates with 30-40% conversion to vanillylmandelic acid. In normal human subjects, plasma free dihydroxyphenylglycol is 200 pg/ml and dihydroxymandelic acid 700 pg/ml. These metabolites provide an index of the activity of noradrenergic neurones in both the peripheral and central nervous systems under a variety of pathophysiological situations. Deuterated methionine administered to controls and patients with altered norepinephrine metabolism will be analyzed for normetanephrine and metanephrine by gas liquid chromatography-mass spectrometry. Changes due to a phenylethanolamine-N-methyltransferase (PNMT) inhibitor will be studied.

PROJECT DESCRIPTION:

The objective of this work was to provide an index of release and turnover of norepinephrine (NE) from catecholaminergic neurones by measurement of catecholamine metabolites in plasma and urine.

The methods used included synthesis of deuterated 3-methoxy-4-hydroxy phenylglycol (MHPG), vanillylmandelic acid (VMA), dihydroxyphenylglycol (DHPG) and dihydroxymandelic acid (DHMA) for assay of these metabolites by gas chromatography-mass spectrometry.

In one aspect of the study, the turnover and distribution of deuterated MHPG was assessed in the monkey and man after intravenous bolus administration. In the monkey, MHPG has a half-life of 40 minutes in the circulation and is rapidly metabolized and excreted as its sulfate and glucuronide conjugates. The relative enrichment of MHPG conjugates is lower than that of free MHPG indicating that 10-30% of the conjugates are derived from the brain. Thirty-forty percent of MHPG is converted to VMA which is found in plasma and urine 5 minutes after administration of the deuterated glycol. The quantitative significance of this metabolic transformation has been underestimated previously, and MHPG metabolism provides an important contribution to urinary VMA excretion.

In another part of the study, methods were developed for the assay of plasma and urinary DHPG and DHMA. The resting level of DHPG in human plasma is 200 pg/ml and of DHMA, 700 pg/ml. It is intended to study the effect of stress and anxiety of decrease states such as hypertension, orthostatic hypotension, depression and other psychiatric disorders on these metabolites and to correlate these measurements with other indices of noradrenergic neuronal activity.

The significance of these studies is that it provides a further technique to examine the activity of catecholaminergic neurones which have been implicated in the pathogenesis of a number of psychiatric diseases as well as disorders of reflex control of the cardiovascular system. In conjunction with the measurement of tissue levels of catecholamines, this methodology promises to further the understanding of catecholamine neurotransmitter mechanisms and their role in disease states.

NE is N-methylated to epinephrine. This reaction is due to the enzyme phenylethanolamine-N-methyl transferase (PNMT) and S-adenosyl-methionine (SAMe). SAMe can be labelled with deuterated methionine. Deuterated methionine will be administered to normal subjects and to patients with central or peripheral disorders of NE metabolism

methylated metabolites in urine examined to determine the rate of methylation of NE to epinephrine. Since NE and epinephrine are rapidly converted to normetanephrine and metanephrine; the incorporation of deuterium into the 3-O-methyl position of these metabolites will be studied also. These studies will be of particular value in patients treated with a PNMT inhibitor.

Preliminary work has been done on the separation and purification of normetanephrine from urine. An acetylated derivative has been prepared and shown to be suitable for analysis by selected ion monitoring on the GLC-MS.

The studies will be extended to examine steady-state level turnover of these metabolites in patients with a range of diseases in which abnormalities of catecholamine metabolism has been implicated.

PUBLICATIONS:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00230-12 LDP

PERIOD COVERED
October 1, 1977 through September 30, 1978

TITLE OF PROJECT (80 characters or less)
Intellective Processes in Young Children: Relationship to Cognitive Functioning in Infancy

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Albert J. Caron	Research Psychologist	LDP NIMH
Other:	Rose F. Caron	Adjunct Professor Department of Psychology	George Washington Univ.

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.65	.15	.50

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of the project is to examine the relationship between information-processing in early infancy and intellective functioning in childhood. The Stanford-Binet IQ scores of 4 to 7-year-old children are being related to their performance on habituation and discrimination-learning tasks at 4 months of age. Results obtained thus far suggest that the association between habituation rate and IQ is constrained by the type of stimulus used in habituation and the sex of the child, with girls showing stronger correlations than boys and the direction of correlation depending on the "Meaningfulness" of the stimulus. Given the difficulties inherent in relating process and outcome variables, the data hold promise for the development of a behavioral assay of cognitive deficit early in life.

PHS-6040
(Rev. 10-76)

Project Description:

Although the measurement of mental abilities in infancy has undergone many changes and refinements, and the available tests have some diagnostic value in the assessment of early deficits, infant test scores have generally not been predictive of later intellectual functioning.General dissatisfaction with the absence of reliable age-to-age correlations of standard infant tests has led many to question their usefulness as an assessment device for cognitive processing ability. Experimental investigations of infant learning and cognition, on the other hand, continue to provide evidence of robust information-processing capacities in infancy. Investigators are impressed, too, with the range of individual differences in performance and with the possibility that these early differences may prove developmentally stable and predictive of future accomplishment. For example, empirical observations suggest that response decrement to repeated stimulation (rate of habituation) in young infants may be related to performance on cognitive tasks such as concept formation and conventional IQ measures in the preschool and early school years. The purpose of the present project is to determine the extent of the relationship, if any, between infant discriminative performance on habituation and learning tasks and measures of intellectual ability in early childhood.

Two areas of activity have occupied the present project during the past six months: (1) completion of the administration of Stanford-Binet and Bender-Gestalt tests to 4- to 7-year-old children who were habituated in visual fixation at 4 months of age to natural and distorted versions of the human face, and (2) adminsitration of the Stanford-Binet to 2-year-olds who were tested in a discrimination learning procedure at 4 months of age.

With regard to the habituation group, we had initially hypothesized that, since rapidity of visual habituation may reflect efficiency of information processing, infants who had habituated most steeply to our face stimuli at 4 months of age would exhibit higher IQ scores later in childhood. To provide as favorable a test of this hypothesis as possible, we compared a subsample of 70 infants whose habituation rates were at opposite extremes, i.e., those who had failed to habituate to our least complicated faces (faces with missing features) versus those who had habituated rapidly to our most complex faces. The IQ differences between these groups, while in the predicted direction (114.6 vs. 116.5, respectively), did not approach significance, with males and females showing comparably non-significant discrepancies.

Although these results indicated that there was no relationship between habituation rate in infancy and childhood IQ, we reexamined the data from another perspective. Our infants had each been habituated to a different version of a schematic face. Some of the stimuli were decidedly facelike or "meaningful." Others were scrambled so that they looked more like samples of abstract art than faces ("meaningless"). Others could best be described as semi-facelike. We hypothesized that whereas rapid habituation to meaningless faces might reflect efficient information-processing, rapid habituation to meaningful faces might index the opposite. We knew from our previous

research (Caron, Caron, Caldwell, & Weiss, 1973) that at 4 months of age infants are just beginning to internalize the full face schema as a consequence of experience in the natural environment. It seemed that an infant who was particularly advanced in this regard might be more likely to notice the "faceness" of our meaningful stimuli, i.e., to assimilate them to his/her already formed schema. This might cause him to fixate these stimuli more intently and thus to retard his rate of habituation. Conversely, a child who was late in developing the face schema, might be less likely to perceive our schematic faces as meaningful stimuli, and would in general habituate to them more rapidly than a more advanced child. If it can be assumed that the earlier an infant develops the natural face schema, the better his information-processing ability, then the correlation between rate of habituation and later IQ should differ depending on the degree of meaningfulness of the habituation stimulus. One would expect the correlation to be negative for our "meaningful" faces, positive for our "meaningless" faces, and somewhere in-between for our semi-meaningful faces.

Overall, the results from the exploration of this hypothesis indicate that for girls slow habituation to meaningful stimuli and rapid habituation to meaningless stimuli at 4 months of age is associated with high IQ in early childhood, whereas for boys these relationships tend, if anything, to be the reverse. Why the male and female groups did not overlap is hard to say, unless the development of the face schema in boys is somewhat retarded relative to that in infant girls.

Forty-two children in the discrimination learning group in infancy have thus far been tested on the Stanford-Binet between 22 and 25 months of age. We shall continue to test more of these infants, but perhaps more important, we shall also try to assess them at an older age when the IQs have stabilized.

The results of the habituation-IQ study are presently being written up for publication.

Publications:

None.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00242-04 LDP

PERIOD COVERED
October 1, 1977 through September 30, 1978

TITLE OF PROJECT (80 characters or less)

The Development of Sensitivity to the Feeling States of Others

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Carolyn Zahn-Waxler	Research Psychologist	LDP, NIMH
	Marian Radke-Yarrow	Research Psychologist	LDP, NIMH
	Robert A. King	Dir., Inpatient Service	Children's Hospital

COOPERATING UNITS (if any)
None

LAB/BRANCH
Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.30	.20	.10

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Very young children's comprehension of others' emotional states and their interactions with persons in distress were investigated. Twenty-four mother-child pairs were studied for a 9-month period. The children were 9, 15, or 21 months old at the beginning of the study. Trained mother observers recorded their children's responses to naturally occurring emotions and to simulated distresses. Between 10 months and 2½ years of age there were identifiable developmental transitions in children's responses to witnessed distress in another person. The repertoire of children 10-12 months is limited; mainly signs of general agitation occur in the presence of another person's emotions. The first responses directed toward the distressed person appear between 12 and 15 months; these are primarily positive physical contacts. An expanded range of responses - including imitation of victim's emotional expression, self-reference, offering of material and psychological comfort - develops between 18 and 24 months. Individual response patterns to distress are also discernible. Developmental changes are discussed in terms of Hoffman's theory of the development of empathy and altruism.

PHS-6040
(Rev. 10-76)

Project Description:

The focus of this research is on developmental changes in very young children's responses to others' emotional experiences, principally in circumstances of distress. The child's attention, emotional arousal, inferences, and actions toward the "victim" and toward the self were investigated. Signs of empathy and compassionate behavior were especially examined.

Three age cohorts, of eight mother-infant pairs each, were selected at 9-10 months, 15-16 months, and 21-22 months of age. The subjects were observed over a 9 month period. The volunteer families were a middle to upper-middle class, suburban sample.

Data were from three sources: (1) Mothers who had been given training in techniques of observation dictated accounts of naturally occurring events in which feelings or emotions were expressed by someone in the child's presence. (2) Mothers also dictated accounts of specified emotional events simulated by them on a predetermined schedule. (3) At three week intervals an investigator visited the home, simulated emotional incidents and observed the mother and child in interaction. Estimates of reliability of reports obtained by comparing mothers' and researchers' reports of simulated incidents indicated generally high interobserver correspondence.

Emphasis in the analysis has been on developmental transformations in children's responses to others' emotional distress. Age changes are discussed within the framework of M. Hoffman's theory of the development of empathy and altruism. The repertoire of the youngest children (10 to 12 months) is limited when confronted with someone's emotions; the central tendency, with little deviation, is agitation evident in facial expression or distress cries. The first responses that are directed to the person in distress appear between 12 to 15 months. These are predominantly forms of positive physical contact. A much expanded range of responses develops between 18 and 24 months. In these responses appear imitations of the victim's expressions, self-referential behaviors seeking assurances from mother, and complexly coordinated offerings to the victim (material, affection, verbal comfort).

In addition to clear developmental lines, individuality in characteristic responses to others' distress were also found. The extent of stability on individual response patterns is being investigated in a follow-up study of these children (see Z01 MH 00273-01 LDP).

A manuscript has been written for publication.

Publications:

Yarrow, M.R. The Measurement of Behavior. In Roche, A. and Himes, J. (Eds.): Anthropology, Growth and Development. University of Arizona Press, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00244-03 LDP

PERIOD COVERED

October 1, 1977 - September 30, 1978

TITLE OF PROJECT (80 characters or less)

A Behavior Profile for Child Psychopathology and Social Competence

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Thomas M. Achenbach	Research Psychologist	LDP	NIMH
Other:	Craig S. Edelbrock	Staff Fellow	LDP	NIMH

COOPERATING UNITS (if any)

25 child guidance clinics and other child mental health agencies

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION

NIMH ADAMHA NIH Bethesda, Md.

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.95	.35	.60

CHECK APPROPRIATE BOX(ES)

X☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

X☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of the research is to develop a standardized behavioral profile for child psychopathology and social competence to be used for research on etiology, treatment effects, epidemiology, and prognosis, as well as for clinical purposes. Parents of disturbed and normal children fill out a behavior checklist describing their child's behavior problems and strengths. Data from the checklists are factor analyzed to identify items that group together within various age and sex groups. The factors are employed as scales of the behavior profile. Empirically obtained distributions of scales on each score provide norms with which the scores of individual children can be compared, and behavior profiles obtained on the same child at different times can be used to assess changes in behavior.

PHS-6040
(Rev. 10-76)

Project Description:

An acute problem in research on the etiology, epidemiology, treatment, and prognosis of child psychopathology is the lack of a standardized and objective system for assessing childhood behavioral disorders. The purpose of the present research is to develop the Child Behavior Profile, an instrument that employs readily obtainable data to portray children's behavioral problems and social competencies in a standardized and objective format.

The data are obtained with the Child Behavior Checklist, which is filled out by parents of children aged 4 to 16 years. Behavior problems (e.g., hyperactivity, disobedience, fears, bedwetting) are scored on a three-point scale according to intensity and frequency. Social competence items include reports of participation in sports, hobbies, and organizations, special skills, number of friends and amount of interaction with them, and school performance. The Behavior Checklist is the product of several pilot editions that have been tried out in child guidance clinics and have been progressively revised on the basis of feedback from clinicians, parents, and paraprofessionals.

The data for the Child Behavior Profile have been obtained from Behavior Checklists filled out by parents of children seen in some 25 mental health settings, plus randomly selected parents of normal children. Factor analysis is employed to identify items that tend to occur together in children grouped by age and sex. Several hundred subjects are included in each sample (e.g., boys aged 6 to 11, girls aged 6 to 11, boys aged 12 to 16, etc.)

The groupings of items obtained through factor analysis are used to define the scales of the standardized profile for each age and sex group, and the empirically obtained distributions of scores on each scale provide norms with which the scores of individual children can be compared. The Profile can be used to classify children for purposes of research on etiology, treatment effects, epidemiology, and prognosis, as well as providing clinicians with a standardized tool for describing and communicating about psychopathology.

Profiles have been completed for both sexes at ages 6 to 11 and 12 to 16, with Profiles for boys aged 4-5 and for girls aged 4-5 expected to be completed in that order. Average one-week test-retest reliability coefficients have been .89 for 6- to 11-year-old boys, .82 for 12- to 16-year-old boys, .88 for 6- to 11-year-old girls, and .90 for 12- to 16-year-old girls. Average interparent agreement coefficients have been .74 for 6- to 11-year-old boys, .79 for 12- to 16-year-old boys, .63 for 6- to 11-year-old girls, and .54 for 12- to 16-year-old girls. Significant stability has also been found in Profiles scored before and after outpatient psychiatric treatment, over a follow-up interval of 18 months. The stability coefficients averaged .63 for 6- to 11-year-old boys, .54 for 12- to 16-year-old boys, .55 for 6 to 11-year-old girls, and .42 for the 12- to 16-year-old girls. Despite decreases in the number of behavior problems reported after treatment, the patterning of behavior remained relatively stable, as reflected in significant Q correlations between the shapes of Profiles obtained at intake and

followup. The editions of the Profile for 4- and 5-year-olds of both sexes are scheduled to be completed during the coming year.

Publications:

Achenbach, T. M. The Child Behavior Profile: I. Boys aged 6-11. J. Consult. clin. Psychol. 46: 478-488, 1978.

Achenbach, T. M., and Edelbrock, C. S. The Child Behavior Profile: II. Boys aged 12-16 and girls aged 6-11 and 12-16. J. Consult. clin. Psychol., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00245-03 LDP

PERIOD COVERED

October 1, 1977 - September 30, 1978

TITLE OF PROJECT (80 characters or less)

Objective identification of the hyperkinetic syndrome

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Thomas M. Achenbach Research Psychologist LDP NIMH

Other: Gerald L. Brown Research Psychiatrist BPB NIMH

COOPERATING UNITS (if any)

Biological Psychiatry Branch, NIMH

LAB/BRANCH Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION

NIMH ADAMHA NIH Bethesda, Md.

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.10	.05	.05

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of the study is to identify objectively the behavioral patterns associated with findings obtained through intensive medical workups of children referred for hyperactivity. Both parents of a referred child independently complete a behavior checklist from which a standardized behavior profile is derived. Degree of inter-parent agreement is computed and the profiles are analyzed in order to classify children according to types of behavior patterns. Classification of children in this way is then compared with ratings of ward behavior, with the outcomes of the medical work-up, and with the effects of stimulant drugs to determine whether the behavioral typology corresponds to characteristics of the children identified clinically.

PHS-6040
(Rev. 10-76)

Project Description:

The "hyperkinetic syndrome" is today the most widely publicized of childhood disorders. There has been a tremendous increase in the number of children labelled as hyperkinetic and a great deal of controversy over such diverse approaches to treatment as chemotherapy, dietary manipulations, behavior therapy, and laissez faire permissiveness. Etiological hypotheses show a parallel diversity, with minimal cerebral dysfunction, allergies, anxiety, inappropriate reinforcers, constitutional characteristics of temperament, and creative nonconformity all being implicated by one authority or another.

One reason for the diversity of treatments and etiological hypotheses is that there is no objective way to define the behavioral phenotype of hyperkinesis. Since most children are from time to time too active for the tastes of their parents and teachers, and since the term "hyperactivity" and even "hyperkinesis", has gained such wide currency, reports of high activity level alone cannot be regarded as pathognomic. Identifying children who share some common etiological factor with respect to activity level or who would benefit from a particular treatment has thus been made difficult by the excessively broad application of the concept of the hyperkinetic syndrome.

In order to determine whether there is an identifiable behavioral phenotype corresponding to a clinical syndrome which may include organic anomalies and which may benefit from chemotherapy, collaborative research is being carried out with Dr. G. L. Brown of the NIMH Biological Psychiatry Branch in connection with a study of children referred for hyperactivity (cf. Biological Psychiatry Branch Project Number Z01 MH 0100-04 BP). Both parents of each referred child independently complete the Child Behavior Checklist (described in report on Project Number Z01 MH 00244-03 LDP). An extensive life history is also obtained and the children are admitted to the NIH Clinical Center for several weeks of medical, behavioral, and pharmacological assessment. Without knowledge of the results of these assessments, we are scoring the behavior profile patterns from the Checklist completed by each parent. Interparent reliability is assessed and the separate profiles as well as a composite profile based on the checklists completed by both parents are analyzed to identify particular types of profile patterns. These are being compared with the results of the clinical work-ups to determine whether the types of profile patterns correlate with differential clinical findings and with ratings of ward behavior. Preliminary findings indicate a clearcut behavioral phenotype of hyperactivity and high correlations between parent ratings on our Checklist and one developed by C. Keith Conners for use in evaluating the effectiveness of drug treatments with children.

If further relationships between the behavior profile patterns and clinical findings are confirmed, the profile patterns can be employed to form homogeneous groups for research purposes. As specific etiologies and/or treatments are discovered for these groups, the profile patterns can be employed by clinicians to guide diagnosis and the choice of treatment.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00246-03 LDP

PERIOD COVERED
October 1, 1977 - September 30, 1978

TITLE OF PROJECT (80 characters or less)

A Follow-up Study of Children Differing in Behavior Problems

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Thomas M. Achenbach	Research Psychologist	LDP NIMH
Other:	Craig S. Edelbrock	Staff Fellow	LDP NIMH

COOPERATING UNITS (if any)

3 Child Guidance Clinics in New Haven and Waterbury, Connecticut

LAB/BRANCH
Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION
NIMH ADAMHA NIH Bethesda, Md.

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.25	.20	.05

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purposes of the research are (1) to determine the stability of behavior profiles based on behavior checklists describing disturbed children, and (2) to determine the prognosis for children differing in initial profile patterns. Parents of children for whom Child Behavior Checklists were filled out at intake filled out Checklists describing their children's behavior problems and social competencies following treatment. Behavior profiles based on the follow-up Checklists are being compared with those based on the initial Checklists to determine the stability and differential prognosis for different types of profiles in various age, sex, and socioeconomic groups.

PHS-6040
(Rev. 10-76)

Project Description:

Effective utilization of mental health services to children is greatly handicapped by the current lack of knowledge about prognosis for various behavior problems. Although the demand for services to children far exceeds the supply, there is no objective basis for selectively assigning treatment to those who will benefit most, because it is not known which children will actually be helped by treatment, which ones will improve without treatment, and which ones will fail to be helped by current treatment approaches.

A primary obstacle to research on prognosis has been the lack of a standardized and reliable diagnostic-classification system for childhood behavior disorders. As described in the report on Project Number Z01 MH 00244-03 LDP the investigators have developed a standardized behavior profile for child psychopathology and social competence. It is expected that children having different profile patterns will differ in their prognosis under various conditions of treatment and nontreatment.

To test both the stability and the predictive power of the profile patterns, a follow-up was conducted of 200 children for whom Child Behavior Checklists were filled out in three clinics at intake. Parents of these children were asked to fill out Checklists describing their children's behavior problems and social competencies following termination with the clinics. Statistical analyses are now being conducted to determine whether children who differed in their initial profiles subsequently differ with respect both to their patterns of problems and competencies and to their general adjustment. Preliminary findings indicate statistically significant stability in the patterns of reported problems despite significant declines in the number of problems reported.

Further analyses are expected to show whether initial profile patterns are differentially related to treatment outcome and whether the patterns are useful for determining prognosis under typical clinic procedures. The findings will also provide the basis for future controlled studies of the effectiveness of various treatments with children having different profile patterns.

Publications:

Achenbach, T. M. The Child Behavior Profile: An empirically based system for assessing children's behavioral problems and competencies. Int. J. ment. Hlth., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00247-03 LDP

PERIOD COVERED October 1, 1977 - September 30, 1978

TITLE OF PROJECT (80 characters or less)

Relations between Cognitive and Behavioral Patterns in Children with Endocrine Abnormalities

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Thomas M. Achenbach	Research Psychologist	LDP NIMH
Other:	Craig S. Edelbrock	Staff Fellow	LDP NIMH
	Roger Johnsonbaugh	Pediatric Endocrinologist	Nat. Naval Medical Center

COOPERATING UNITS (if any) National Naval Medical Center

LAB/BRANCH Laboratory of Developmental Psychology

SECTION --

INSTITUTE AND LOCATION NIMH ADAMHA NIH Bethesda, Md.

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.10	.05	.05

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Behavior checklists and measures of cognitive functioning are being obtained on normal controls and children who have thyroid and sex hormone abnormalities in order to determine whether hormone levels affect patterns of cognitive and behavioral adaptation and the relationships between them. The study is designed to test the hypothesis that increases in sex hormone level affect patterns of cognitive functioning by improving automatization abilities at the expense of ability to engage in complex perceptual restructuring. It is also designed to determine whether premature craniosynostonis noted in hyperthyroid children interferes with cognitive functioning.

PHS-6040
(Rev. 10-76)

Project Description:

One of the most neglected aspects of research on childhood behavior disorders is the interface between cognitive functioning and maladaptive behavior. Research and theory tend to focus on cognitive disabilities as one substantive area and "emotional" problems as a separate substantive area. However, from a developmental perspective, cognitive functioning is intimately interwoven with the social, biological, and emotional aspects of adaptive processes. On the one hand, a child's level of cognitive development is likely to set limits on the types of adaptive strategies available to him. On the other hand, social, biological, emotional, and motivational factors are likely to influence the degree to which a child makes use of his cognitive capacity.

Work by other investigators has suggested that sex hormone levels affect the action of neurotransmitters in the brain, and that these, in turn, affect the patterning of cognitive abilities. In particular, it has been found that high levels of sex hormones, especially estrogens, are positively correlated with performance on automatization tasks requiring quick, accurate responses, but are negatively correlated with performance on tasks that require complex perceptual restructuring. Our study is designed to test this hypothesis by comparing cognitive functioning and behavioral patterning before and after treatment in children who receive sex hormone supplementation for various endocrine abnormalities. Children with thyroid abnormalities are also being studied because clinical observations have revealed premature craniosynostonis in these children, and it is important to determine whether cognitive and behavioral patterns are being affected by this condition. The children and their sibling controls are recruited by Dr. Roger Johnsonbaugh through his pediatric endocrinology service at the Naval Medical Center.

Our measures of cognitive functioning include the Wechsler Intelligence Scale for Children (WISC), the Embedded Figures Test, a measure of speed of color naming, and a measure of speed of object naming. Differences between perceptual restructuring measures (WISC, Block Design, Embedded Figures Test) and automatization measures (color naming, object naming) will be compared at two testings to determine whether changes in hormone levels after medication cause changes in cognitive patterning that do not occur in normal, unmedicated control subjects.

Preliminary findings indicate some differences in cognitive patterning between children with thyroid abnormalities and their normal controls, but not enough subjects have yet been tested to determine the generality of these differences or to draw conclusions about the effects of sex hormones.

Publications:

Edelbrock, C. and Sugawara, A. I.: Acquisition of sex-typed preferences in preschool-aged children. Dev. Psychol., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00248-02 LDP

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Sources of Variability in Children's Sensitivity to Others

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	David E. Barrett	Staff Fellow	LDP NIMH
	Marian R. Yarrow	Chief, LDP NIMH	LDP NIMH
OTHER:	Claire Horowitz	Psychologist	LDP NIMH

COOPERATING UNITS (if any)
Green Acres Summer Day Camp

LAB/BRANCH
Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.40	.30	.10

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this study is to investigate individual differences in children's sensitivity to others, and to examine sources of this variability. The study involves the naturalistic observation of children at a summer day camp, as well as interviews with each child to study his/her social inferential abilities. Specific issues investigated include (1) relations between aggressive and assertive behaviors and prosocial behaviors, (2) the dimensionality of prosocial behavior, specifically relations among different types of prosocial acts, (3) the possible mediating effects of role-taking and inferential abilities with regard to the relations between children's past interpersonal experiences and their later social behaviors, (4) sequential dependencies involving aggressive and prosocial behaviors, i.e., the facilitative or inhibitory (accelerating or decelerating) effects of expressed aggression with regard to later prosocial behavior. In addition, sex differences in aggression as a function of setting, mode of aggression, and sex of target are investigated.

PHS-6040
(Rev. 10-76)

Project Description

The present study examines sources of variability in prosocial behavior in young children. Proposed and approved as a continuation of an earlier study on the development of prosocial behavior (Project #73-M-02, Developmental Studies of Altruism), the current study focuses on research issues raised by results of the earlier study; specifically, questions concerning the dimensionality of prosocial behavior, behavioral correlates of prosocial behavior, and the role of social inferential ability in the development of prosocial behavior. In addition, the study has examined sequential constraints in children's social interaction, particularly with respect to prosocial and aggressive behaviors, and sex differences in aggression.

Subjects were 79 children, ages five to eight, attending a summer day camp. Each subject was observed eight times over the course of the six week period, in both free play and camp activity. During each of the 15 minute observation sessions, observers recorded the child's social behavior on a precoded form. Inferential ability was assessed in an individual interview in which the child interpreted a series of videotaped social interactions.

Findings concerning correlations between prosocial behavior and aggression were reported in the 1975-1976 annual report as were results of an analysis concerning the mediating role of social inferential ability in the prediction of prosocial behavior. Results relating to the issue of sequential constraints were summarized in last year's annual report.

The final set of analyses has focused on the issue of sex differences in aggression. Despite the consistency of empirical findings indicating that human males are more aggressive than females, few naturalistic, observational studies on children's aggression have employed methods that would allow for assessment and control of contextual factors which might mediate the relationship between sex and aggression. In the present study several factors thought to influence the magnitude of sex differences in aggression were explicitly considered. Children were observed in different settings, some closely supervised, some less structured. Provocations to aggression were accounted for and the sex of the target of aggression was identified. Finally, analyses controlled for sex differences in frequencies of non-aggressive social initiations.

Results indicated that boys had significantly higher raw frequencies of aggression to boys than did girls, but the magnitude of the difference was reduced when social initiations were controlled. In contrast, sex differences in aggression to girls were increased when social initiations were controlled, with males again the more aggressive. Sex differences in aggression were more marked in moderately adult-regulated settings than in a free activity setting. Effects of controlling for provocation were complex, depending on the mode of aggression and sex of target.

In summary, the results of the present study identify several contextual factors which may affect the magnitude and even the direction of observed sex

differences in children's aggression. The inferences one draws about sex differences in aggression are likely to be influenced by such features of the measurement paradigm as the observation setting, the available targets of aggression, and controls for provocation to aggression and frequency of non-aggressive social initiations. Clearly, any conclusions concerning the biological or experiential bases of sex differences in aggression must be based on studies which have carefully considered the setting events and mediating conditions which may serve to enhance or minimize such differences in behavior.

Publications:

Barrett, D. E. Relations between aggressive and prosocial behaviors in children. J. Genet. Psychol., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00250-03 LDP

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)
Information Processing in Premature and Full-Term Infants at the Perinatal Period

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Sarah L. Friedman	Research Psychologist	LDP NIMH
OTHER:	Blanche S. Jacobs	Psychologist	LDP NIMH
	Milton W. Werthmann	Chairman, Dept. of Perinatal & Neonatal Pediatrics	Washington Hospital Center, Wash. D.C.

COOPERATING UNITS (if any)
Washington Hospital Center, Washington, D.C.

LAB/BRANCH
Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.78	.83	1.95

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In this study precursors of cortical function and temperament are compared in infants varying in gestational and medical status at birth. The infants who are being studied include healthy as well as previously sick prematures at their expected date of delivery, healthy full-term neonates and post-term neonates. Cortical function is evaluated by measures of habituation, dishabituation and transfer of learning in response to tactile, auditory and visual repeated stimulation. Temperament is assessed by measures of states of consciousness, the range and quality of activity while the babies are free of apparent discomfort and by the persistence of spontaneous and elicited crying in the face of experimentally designed attempts to quiet the infants. The results are expected to identify factors which place children at greater than average risk for later intellective and behavioral dysfunction. The results also provide information about the importance of maturation and medical integrity for the function of different sensory modalities and the organization of behavior.

PHS-6040
(Rev. 10-76)

Project Description

Most pregnancies last between 38 and 42 weeks. Some, however, terminate much earlier or later. Medical technology has made it possible for infants born as early as at 28 weeks or as late as at 50 weeks of gestation to survive. This is certainly an impressive medical accomplishment; yet, the psychological outcomes of too short or too long periods of gestation are not fully understood. It is not obvious that the central nervous system of a premature organism is ready to benefit from extrauterine stimulation. Nor is it obvious that a postmature organism has not suffered because of its not being exposed to an extrauterine environment at a possibly critical period for some aspects of development. Birth not at term is often but not always associated with medical complications. This raises the question about the role of illness in early development. It is possible that a newborn who spends much energy in a struggle for biological survival has very little energy left for psychological development.

Evidence from late infancy and childhood indicates that children who were not born at term are at a greater than average risk for intellective and behavioral deficits. In this study we explore some precursors of intellective and behavioral deficits in early infancy -- in the days after birth or at the expected date of birth (in the case of prematures). Knowledge of specific weaknesses and strengths at this early stage should make it possible rationally to plan early intervention programs to stimulate those aspects of development which show deficits due to birth not at term.

The psychological functions which are studied are of two classes: information processing as an index of the degree of cortical maturity and integrity, and temperament. Information processing is assessed in the tactile, auditory and visual modalities. In the examination for tactile information processing, the infant's cheek is stroked repeatedly by one of two non-noxious stimuli (a nylon filament applied in two directions) for 2-second periods, with a random interval of 15 to 25 seconds. When the baby has habituated to a predetermined criterion, the other stimulus is applied until the baby habituates to it. The data provide measures of habituation, response to novelty (dishabituation) and a comparison of the ease of habituation on the second occasion relative to the first (transfer of learning). In the auditory information processing examination the infant is presented with one of two tones (500 cps; 1500 cps at 80 dB). The procedure and measures will be equivalent to those described for the tactile modality. In the visual information processing examination each of the two stimuli (revolving, lighted, differently colored plexiglass boxes) are presented for 30-second periods starting with the infant's first look at the stimulus. The inter-stimulus interval is 10 seconds. The procedure and measure are similar to those employed in the tactile and auditory procedures.

Temperament is assessed by the range and quality of the babies' behaviors when they are free of discomfort as well as by the resistance of their spontaneous and elicited crying to comforting. The temperament assessment is

conducted when the baby is satiated and quiet. During 18 minutes the baby is observed for periods of 5 seconds to be followed by 10-second periods in which the experimenter notes her observations. The observations include the infant's state of consciousness, his/her body, limb, and facial movements as well as looking activity and vocalizations. If the baby cries spontaneously an experimenter intervenes in a progressively soothing manner in an attempt to comfort the baby. The intervention and the infant's responses are coded. The temperament assessment terminates with the coding of intervention following elicited crying. Crying is elicited by flipping a finger against the infant's sole of the foot. We are in the process of data collection. Eighty babies are expected to comprise the final sample. Over half of this number has already been tested.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00251-02 LDP

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Effect of Contingent Maternal Responding on Infant Crying

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Jacob L. Gewirtz, Ph.D. Research Psychologist LDP NIMH

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION
NIMH ADAMHA NIH Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.30	.15	.15

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Will contingent maternal responding to nondistress infant crying lead to a subsequent decrease in that crying (as the 1972 Bell and Ainsworth conclusion has led many to expect) or will it lead to a crying-rate increase (as would be expected under instrumental learning theory)? Subjects were 12- to 14-week-old normal infants. Infant lay on floor, alone in room with door ajar. The cry response was defined as a 5-sec. shrill or whiney vocalization, accompanied by a facial grimace. Under noncontingent responding, mother entered routinely at 1-min. intervals to visit her infant, provided s/he was not crying. Under contingent responding, mother's visits were contingent on infant's 5-sec. cries. Number of maternal 10-sec. visits (i.e., stimulus density) was comparable under both treatments. Mother acted naturally, but did not pick up infant. Compared to mothers' noncontingent responding, their contingent responding systematically increased their infants' mean crying rates ($p<.012$). Therefore, maternal responding contingent on infant nondistress cries increased the cry rate as expected under instrumental-learning theory. This result has implications for the caregiver management of infant crying.

PHS-6040
(Rev. 10-76)

Project Description:

This research focuses on infant crying that is communicative or instrumental and not elicited by physical distress. It attempts to determine some features of a mother's responding to her infant's nondistress crying that foster or maintain that crying and those that minimize or preclude that crying. A second objective is to use the findings of the research to make recommendations to caregivers about procedures for minimizing nondistress infant crying in the first year of life.

In early life, physical distress underlies the crying termed "unconditioned," "expressive," or "respondent." On that basis alone, infant crying has a unique potential for controlling caregiver behavior. However, there is no apparent unlearned basis for the infant crying termed "operant," "instrumental," or "communicative," which constitutes an important feature of early communication of infants with caregivers. An understanding of how crying patterns are acquired offers a unique basis for understanding how attachments evolve in the child. At the same time, there has been recent controversy about how caregivers would respond to infant crying to alleviate bodily distress while not fostering crying and "spoiling" the child. This project was directed to determining whether and how early infant operant/communicative crying/fussing could be controlled by maternal responding to it. Will contingent maternal responding to nondistress crying lead to a subsequent decrease in that crying (as the 1972 Bell & Ainsworth conclusion has led many to expect) or will it lead to a crying-rate increase (as instrumental-learning theory would predict)?

Mothers brought their infants to the laboratory daily at a time judged best for the infant. To preclude physical distress-induced crying, infants were studied only when they had been recently fed and diapered, had slept routinely, and did not appear tired or ill. The criterion cry response (fuss or cry) unit was defined as a shrill or whiney vocalization, 5 seconds long, accompanied by a facial grimace. Observer agreement on cries was high. Daily sessions were 16 minutes long, but were terminated prematurely when an infant cried/fussed without pause for 1 minute, or fell asleep.

With its mother not in view outside the experimental room, each infant was positioned on the floor, alone in the room. Under noncontingent maternal responding, a mother routinely entered the room to visit her infant at one-minute intervals, provided the infant was not crying. Under contingent maternal responding, each mother's visit began immediately after her infant had cried for 5 seconds. The standard maternal response was a 10-second visit to the infant. Each mother was asked to act naturally, by touching, nuzzling, patting, squeezing, kissing, and/or talking to her infant, but not picking up the infant. She was signalled to enter the room, and to leave it after 8 seconds of a visit. Upon departing, she activated an overhead mobile gently. She routinely checked her infant's diaper and, if diapering was indicated, did so while the session-duration timer was stopped. The number of visits was comparable under both noncontingent and contingent treatments. Hence, an outcome difference could not be explained as due to the differential elicitation of crying by different numbers of maternal visits.

Mothers and their 12- to 14-week-old normal, full-term, infants served as subjects. Seven subjects received first the noncontingent and second the contingent treatment; and 3 subjects received the treatments in reverse order. When rate of crying had stabilized under the first treatment, the infant was shifted to the second. Thus, each infant served as his/her own control in a repeated-measures design. Excluded from the sample were infants exhibiting very-high, very-low, or very-variable crying rates during the first four sessions. On this basis, it was necessary to discard two infants for every infant qualifying.

Crying rate (the number of cries per minute) was the dependent measure for each daily session. The final session of the first treatment condition occurred when the cry-rate scores had stabilized. (The median number of sessions required for the cry rate to stabilize was 4 for each of the two treatment-order groups.) Comparisons were made between the final three sessions of the first treatment and the initial three sessions of the second treatment. There were no differences in means between the infants exposed first to non-contingent stimulus provision and second to contingent stimulation, and the infants receiving the reverse order. When the two treatment-order groups were pooled, higher and increasing crying-rate means were found across sessions under contingent stimulation compared to the lower and decreasing means under noncontingent stimulation ($p<.012$). Therefore, maternal responding contingent on nondistress infant cries increased the rate of that response class relative to noncontingent maternal responding, in keeping with the expectation under instrumental-learning theory.

On the basis of this result, a recommendation might be made to caregivers of infants exhibiting intermediate levels of crying: If confident that they could distinguish physical-distress-induced crying from other crying of their infant, they might repond to alleviate the conditions underlying the distress crying but not respond to its other crying. At the same time, they might move in the direction of responding contingently to the beginnings of such acceptable communicative behaviors as vocal and other noncrying expressive responses.

Having been completed, this project is now closed.

Publications:

Gewirtz, J. L., & Boyd, E. F. Does maternal responding imply reduced infant crying?: A critique of the 1972 Bell and Ainsworth report. Child Development, 1977, 48, 1200-1207. (a)

Gewirtz, J. L., & Boyd, E. F. In reply to the rejoinder to our critique of the 1972 Bell and Ainsworth report. Child Development, 1977, 48, 1217-1218. (b)

Gewirtz, J. L. Contingent maternal responding increases infant crying. Child Development, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00253-03 LDP

PERIOD COVERED
October 1, 1977 - September 30, 1978

TITLE OF PROJECT (80 characters or less)

Behavioral Studies of Seriously Ill Children

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Elizabeth J. Susman	Visiting Fellow	LDP NIMH
OTHER:	Albert R. Hollenbeck	Staff Fellow	LDP NIMH
	Stephen P. Hersh	Psychiatric Consultant to Pediatric Oncology Branch	NCI
	Arthur S. Levine	Chief, Pediatric Oncology Branch	NCI
	Philip A. Pizzo	Senior Investigator	NCI

COOPERATING UNITS (if any)

Pediatric Oncology Branch, National Cancer Institute

LAB/BRANCH
Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION
NIMH ADAMHA NIH Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.09	.70	1.39

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Physical well-being is an assumed, though generally unmeasured, condition in studies of the child's psychological development and behavior. There is little developmental inquiry concerning the effects of loss or absence of physical well-being on child development, despite the reality that acute or chronic ill health from disease or accident is, to a great or small degree, part of the life of every child. This project investigates the psychological and social impact of serious illness (cancer) on children and those in their social network (namely their families). Areas of research include coping abilities, the influence of the social system of the child and the mutual influences of the stressed children and those of his social network. Observations, interviews, and parent and nurse ratings are being employed to investigate these phenomena.

PHS-6040
(Rev. 10-76)

Project Description:

The purpose of this project is to assess the psychological impact of a serious illness (cancer) on children, with particular emphasis on the child's understanding of the illness, the child's subjective experiences associated with illness, and the child's social behavior with significant others.

The research participants are patients on the Pediatric Branch of the National Cancer Institute. The sample chosen for study is a group of children with metastatic pediatric solid tumors and lymphomas that had failed to respond to more conventional therapeutic regimen. These children receive intensive chemotherapy. Such therapy is very toxic and can lead to life-threatening complications. Research participants are randomly assigned to Laminar Air Flow units or the general pediatric oncology unit for a period of five to seven weeks while they are receiving chemotherapy. The purpose of Laminar Air Flow rooms is to provide a protective germ free environment.

Study A. Changes in conception of illness, social behavior, and mood states of children in a protected environment

Research participants are children, 9 to 21 years, 14 assigned to Laminar Flow units, and four assigned to the general pediatric oncology unit. In the Laminar Flow units, children are deprived of important social contacts--parent, siblings, and peers--as well as the normal sources of stimulation in the environment. The research was designed to explore the child's and parent's conception of the illness--its cause, course, and prognosis--and how does this change over time; the relation between the child's conceptions of the illness and emotional states and social behavior during hospitalization; the child's concerns about death; and the role parents and medical personnel play in providing comfort and support to the distressed child.

Multiple methods were used for data collection: systematic observation, interviews, and parent and nurse ratings. Because the research issues are questions of change and interaction, regular and extensive sampling of behavior was done on a continuing basis throughout the period of hospitalization. Behavioral data will be analyzed in conjunction with physiological measures of clinical status.

Children's knowledge of the disease and treatment clearly relate to the stage of cognitive development. For older children, failure to assimilate an accurate and sophisticated understanding of the illness appears to be related to the child's sense of futility about the future, and his sense of lacking control of what happens in relation to treatment and hospital routine. Children of all ages place an inordinate degree of hope in the remission-inducing potential of chemotherapy. Their future orientation is toward living and pursuing age-appropriate activities and engaging in social relationships with peers. The possibility of death is infrequently mentioned. Children are as concerned with age-appropriate issues as they are about their disease and treatment. The children may mention that death is a possibility but their main concern is with the task of living.

Social interactions between child and caregivers change as the child approaches death. There is evidence that mothers withdraw as the child becomes sicker. Mothers initiate fewer behaviors toward the child even though the child may continue to initiate at the same rate. Physical withdrawal appears to be a concommittant of psychological withdrawal which may be necessary in order to protect themselves from the trauma of the child's death. Nurses continue to engage in social interactions with the dying child; their interaction is mandated by the child's pressing medical needs.

Data collection is completed and data are now being analyzed.

Study B. Behavioral observations of young children in isolation, deprived of maternal contact

Research evidence from many sources indicates that human infants need close physical contact with another human and contact that consists of meaningful auditory and visual stimulation. Recent animal studies on monkey mother-infant separation have identified physiological changes occurring in the infant almost immediately after separation from the mother. Such changes consist of decreased body temperatures and heart rate. Behavioral signs of clinical depression (inactivity and fetal posturing) in the infant monkey emerge later. These effects appear to be most deleterious when visual contiguity with the mother was maintained.

The Laminar Air Flow environment is an extreme situation of mother-child separation that is analogous to experimental studies of monkey mother-infant separation. Three boys and one girl ranging in age from 18 months to 4 years were research participants. Each was in Laminar Flow for 4 to 14 weeks while undergoing chemotherapy for malignant tumors. Specific research questions focus on mother-child separation during therapy. (1) How does separation per se influence observable behaviors normally associated with childhood (levels of play, aggression, affect); (2) what are the physiological concommitants related to separation; (3) are there cumulative psychological effects due to separation (e.g., prolonged bouts of anxiety or depression).

Videotaped observations were collected at two points, morning and afternoon, four times a week, during the period of the child's hospitalization. These observations were coded on dimensions of the child's role (initiator-receiver), behavior content, communications, and affect. Physiological measures of heart rate, blood pressure, body temperature and respiration were obtained four times daily. Analyses are in progress but results to date indicate that children were generally physically active and engaged in a variety of vocal behaviors such as crying and talking; play remained relatively constant throughout the treatment. Passivity gradually increased during treatment and then declined after discharge from the Flow room. The increases and decreases in particular behaviors do not appear to coincide with particular aspects of the medical treatment. Amount of behavioral output and the complexity of behavior rose during the first few weeks of treatment then declined during later weeks of treatment.

Of particular interest from the physiological data is that body temperature dropped below normal in the first 24 hours after separation, a pattern also observed in infant monkeys. High temperatures during the remaining period of isolation were a result of chemotherapy.

In general, the children's behavior trends support Reite's primate data and his notion of an agitation-depression syndrome. While these data differ from the primate findings in terms of the exact timing, they nevertheless follow a descriptive sequence of agitation followed by depression. The parallel finding for body temperature underscores the seriousness of maternal separation. However, the relationship between physiological and behavioral indices needs further exploration. In future analyses we hope to identify patterns of functional vs. dysfunctional behaviors which have direct implications for intervention strategies. Videotaped observations are being analyzed further for patterns of behavioral changes in relation to physiological data and clinical states.

Further reports are being prepared.

Publications:

Hollenbeck, A. R. & Susman, E. J. Behavioral studies of young children in a germ-free environment. Presented at the annual meeting of the American Association for the Advancement of Science, February 1978, Washington, D. C. In Abstracts of Papers, AAAS, 78-2, 150, 1978.

Pizzo, P., Levine, A., Simon, R., Marshall, D., Susman, E., Magrath, I., & Robichaud, K. Utility of Laminar-Flow Rooms (LFR) in the delivery of intensive chemotherapy to children with refractory malignancies. Proceedings of the American Association for Cancer Research, April, 1978.

Susman, E. J., Hollenbeck, A. R., Nannis, E. G., Strope, B. E., Hersh, S. P., Levine, A. S., & Pizzo,P.A. Social interactions between children with cancer and their caregivers: A methodology for systematic observation in a hospital setting. In Bugen, L. (Ed.): Death and Dying: Theory, Research, and Practice. Dubuque, Iowa: W. C. Brown, Inc., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00255-02 LDP

PERIOD COVERED

October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Maternal Imprisonment: Psychological Effects on Children

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Velma LaPoint, Ph.D.	Staff Fellow	LDP, NIMH
Other:	Marian Radke-Yarrow, Ph.D.	Chief, LDP, NIMH	LDP, NIMH
	Gale Inoff	Psychologist	LDP, NIMH
	Beverly Fairley	Research Assistant	LDP, NIMH

COOPERATING UNITS (if any)

Maryland Division of Corrections, Department of Public Safety, Baltimore, Md. Institute of Law Enforcement and Criminal Justic, Law Enforcement Assistance Administration (LEAA), Department of Justice, Washington, D. C.

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION

NIMH ADAMHA NIH Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.90	1.15	1.75

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The loss or lack of stable and supportive parenting is assumed to place children at risk for the development of mental health problems. When the reasons for inadequate parenting are themselves indicative of psychological problems of the parents, risk for the child is compounded. Such is the condition of children whose mothers are imprisoned. The purpose of the study is to identify psychological consequences for these children. The relationship between mother and child, the child's attitudes toward self, mood and emotional states, and the child's interpersonal relationships with peers and adult authority figures are studied. The research participants are 40 to 60 incarcerated mothers and their children, and equal numbers of mothers and children in community living single-parented families. The groups are comparable racially (Black) and socio-economically. Data are obtained in four settings: mother groups, caretaker settings, mothers with their children, and experimental child groups. Secondary data are obtained from the schools.

PHS-6040
(Rev. 10-76)

Project Description

The loss or the lack of stable and supportive parenting is assumed to place children at risk for mental health problems. When the reasons for inadequate parenting are themselves indicative of psychological problems of the parents, risk for the child is compounded. Children whose mothers are imprisoned constitute such a group at risk. An estimated 157,000 American children, in any one year, are in this group. The number of children who grow up with this experience are many multiples of this number.

The purpose of this study is to identify psychological consequences for children of mothers' imprisonment. Four aspects of consequences are examined: the relationship between mother and child; the child's moods, emotions, and attitudes toward self; the child's interpersonal relationships with peers and adult authority figures; and the child's abilities to cope with daily demands of living.

The subjects are 40 to 60 imprisoned mothers at the Maryland Correctional Institution for Women, Jessup, Maryland and their children. The children are presently living with substitute caregivers, and are between five and twelve years old. Two comparison groups of community-living, single-parented families, comparable racially and socio-economically but without histories of institutionalization, are also being studied. One group is made up of 30 single-parented families without involvement with law enforcement authorities, and the other is composed of 30 probation mothers and their children. Data are obtained in four settings: mother discussion groups, caretaker settings, mother and child groups during visits to the prison, and experimental child groups. A secondary data source is school records of children's academic and behavioral performance.

Data obtained on mothers pertain to their roles and attitudes toward their children, and their attitudes toward themselves as mothers. Mothers are observed in small group discussions, in interaction in small groups comprised of other mothers and children, and in standard dyadic situations with their child.

The child is seen initially at home with the caregiver. During the home visit the study is explained, and observations are made of the conditions of the home as well as of adult-child interaction. The primary data on the child are observations of interactions with mother in quasi-experimental and in naturalistic settings, and observations of behavior in two experimental groups. The groups are composed of six to eight boys and girls, ages 5 to 8, and 9 to 12. The protocol for the child groups is a programmed schedule of groups and individual activities designed to permit assessments of each child's interpersonal relationships, emotional state, self-confidence, deployment of attention, and impulse control.

On two half-day visits to the prison, behavior of mother and child is observed in certain significant moments such as during the child's arrival and departure from the prison, during full interaction, and during controlled dyadic situations.

The same general sequence of data collection and procedures is followed for control groups with obvious changes in locales.

Findings from this study will provide information about children's coping with personal stress, and about factors influencing the coping process. The findings also have implications regarding policies for imprisoned mothers and their children. A wide range of correctional policies is being considered, yet there is little knowledge about the nature of the mother-child relationship of imprisoned mothers or about the effects of mother's imprisonment on children.

Data collection is in progress.

Publications

LaPoint, V. Mothers Inside, Children Outside: Some Issues Surrounding Imprisoned Mothers and Their Children, 1977 Annual Proceedings of the Congress of Corrections, Maryland: American Correctional Association, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00256-03 LDP

PERIOD COVERED

October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Expressions of Affect: Children's Age and Sex-role Expectations

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Karabelle A. L. Pizzigati	Staff Fellow	LDP NIMH
Other:	Charles F. Halverson, Jr.	Professor	Dept. of Home Economics, Univ. of Georgia, Athens

COOPERATING UNITS (if any)

Green Acres School, Rockville, Maryland

LAB/BRANCH

Laboratory of Developmental Psychology

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INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.80	.60	.20

CHECK APPROPRIATE BOX(ES)

[X] (a) HUMAN SUBJECTS [ ] (b) HUMAN TISSUES [ ] (c) NEITHER

[X] (a1) MINORS [ ] (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

While assumptions about appropriate sex-role behaviors are culturally acknowledged, little is known about the development of differential perceptions of sex-role personality dimensions. Children's and teachers' judgments with regard to sex-role-related personality traits were investigated from a developmental perspective. Children, ages 5-14 years, viewed videotaped skits portraying traits stereotypically associated with one sex or the other (e.g., gentleness, stoicism). The children evaluated themselves, considered peers in terms of the traits portrayed, and assessed the social desirability of the traits. Teachers rated the children on the traits and on aspects of social ease (e.g., nervousness). The findings indicated some instances of sex-role stereotyping, but the degree varied, depending on the child's age, sex and trait being considered. Stereotyped responses occurred more often for boys than for girls, increasing with age. Other aspects of gender and the social desirability of the traits influenced the children's judgments of themselves and their peers. While teachers evaluated the children similarly, few relationships were found between teachers and children's perceptions.

PHS-6040
(Rev. 10-76)

Project Description:

The purpose of this project was to investigate the development of children's perceptions of sex-role-related personality traits. The importance of the gender dimension in children's perceptions of themselves and their peers was studied. Also, teacher ratings of the children on the personality traits were compared with the children's evaluations of themselves. The traits selected for study were gentleness, fickleness, emotional expressiveness, dependency, nurturance, stoicism, messiness, assertiveness, independence, and boastfulness. Evidence from the literature and from pilot data suggest that these traits relate to sex-role attitudes.

The sample consisted of 150 middle and upper-middle class elementary school boys and girls attending a private school. Each participant individually viewed 10 videotapes portraying the traits listed above. For each trait, the children were asked to describe the portrayal, evaluate themselves in terms of the trait portrayed, nominate two peers who exhibit the trait, and evaluate the social desirability of the trait. After all children were tested, teachers in each class rated their students on the ten personality traits and on dimensions of social ease. There were few consistent differences between boys and girls in how they viewed themselves and their peers. The largest and most consistent differences between boys and girls were found for two traits stereotyped as feminine -- nurturance and gentleness. Few differences appeared for the masculine traits.

Sex-role stereotypes appeared more salient for the boys than for the girls. While girls showed little change in their self ascriptions to the masculine and feminine traits from ages 5 through 13, boys increasingly described themselves in masculine terms and decreasingly described themselves in feminine terms. Girls generally appeared to hold more flexible attitudes about themselves and their peers in regard to the traits than did boys. Girls were more likely to ascribe to both masculine and feminine traits than were boys. Also, when they considered their peers in terms of the traits, girls tended to consider both sexes (i.e., to nominate one boy and one girl) whereas boys tended to nominate one sex or the other more often for a given trait.

Sex-role stereotyping was not the only factor which affected the children's responses. Gender influenced the children's nomination of peers. Children overwhelmingly tended to nominate children who were the same sex as themselves, regardless of the trait. This suggests that sex is important when one thinks of peers. However, it probably reflects sex segregation of friendship groups rather than sex-role stereotyping of personality traits.

The social desirability of the personality traits also proved to be an influential factor in the children's judgments of themselves. The children's self-judgments related to their judgments of social desirability most strongly at the youngest age period when the children appeared most egocentric and concrete in style. Social desirability and self ascription also were strongly related for the oldest, pubescent group for whom sex-role and self-identity

issues are crucial. Social desirability was least important for the middle age years. The teacher ratings showed only few and moderate relationships with the children's judgments of themselves and their peers. For the children's judgments and for the teachers' ratings, a feminine dimension, including gentleness and nurturance, discriminated between boys and girls. A masculine factor was not identified for the teachers ratings. This study elucidates the course of sex-role development for boys and girls, and documents some of the situational and socialization factors that seem to affect children's orientations to those around them.

The results are being prepared for publication.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00257-02 LDP

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Effects of CNS Treatment on Intellectual Functioning of Children with Leukemia

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Howard A. Moss	Research Psychologist	LDP NIMH
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	Philip A. Pizzo	Senior Investigator Pediatric Oncology Branch	NCI
	Nili P. Ramu	Clinical Associate Pediatric Oncology Branch	NCI
	Stuart Siegel	Chief, Div. of Hematology-Oncology	Los Angeles Children's Hospital, Los Angeles, California

COOPERATING UNITS (if any)
NCI
Los Angeles Children's Hospital

LAB/BRANCH
Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.39	.50	.89

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The effects of CNS treatment (cranial irradiation and intrathecal chemotherapy) on the cognitive and perceptual functioning of children with leukemia are investigated. One study compares a group of leukemic children in remission with a matched group of sibs on measures from a battery of psychological tests (Wechsler, Bender-Gestalt and Graham-Kendall). These groups are followed-up 12-15 months after the initial assessment. A sample of Ewing sarcoma patients and their sibs and a sample of cystic fibrosis patients and their sibs are also being assessed in these procedures to control for effects other than CNS treatment. Computerized tomographic scans on the leukemia patients as a measure of change in brain structure will also be related to their intellective functioning. A second study deals with another sample studied prospectively. In this instance children in remission from leukemia are evaluated on the same battery of psychological tests as in the first study. They are tested just prior to prophylactic CNS treatment and are re-evaluated one year later to determine if any possible brain changes brought on by the CNS treatment have resulted in learning disabilities and diminished intellectual functioning. A normal control group will be tested at both points in time in order to check for testing effects.

Project Description:

The chance of survival from childhood leukemia has increased greatly over the past 10 years because of the utilization of new treatment procedures. The main change in treatment that has led to this improved outlook is the inclusion of prophylactic measures consisting of a series of cranial irradiations and intrathecal (spinal) injections of powerful chemical agents. These measures are aimed at destroying any stray leukemic cells in the brain and central nervous system.

The purpose of the proposed research is to determine if CNS treatment has a deleterious effect on intellectual functioning and to evaluate the nature and extent of these effects. Two studies are in progress. One study involves evaluation of intellectual functioning only after CNS treatment (post-treatment study); in the other study intellectual performance prior to CNS treatment is compared to performance subsequent to treatment.

Because of the age range of our subjects the three Wechsler Intelligence Tests are used: WPPSI (ages 2½-6), WISC-R (ages 6-16), WAIS (over 16 years of age).

Study I. Post-treatment study

The sample consists of 36 children who have been in remission for 2 to 5½ years from acute lymphocytic leukemia (ALL), and a sibling control group. The mean age of the children at the time of assessment was 11 years with a range of 4 to 23. All children were administered a Wechsler Intelligence Test, the Graham-Kendall and the Bender-Gestalt. This battery will be re-administered to both groups approximately 12-15 months after the initial testing in order to determine if there is any progressive decline in abilities. Readministration of the tests to the control group provides an estimate of test practice effect.

For all comparisons the sibling group performed at a higher level of functioning than the patients, with most of the differences between group being statistically significant. The mean Full Scale IQ of 99 for patients and 112 for siblings indicates that the patients are operating at an average and the siblings at a bright average level of intellectual functioning.

A hypothesis which we investigated was that although the damage from the CNS treatment might not have greatly diminished mental abilities already present at the time of treatment, it might have interfered with the acquisition of new skills and the mastery of higher levels of mental functioning that ordinarily would be consistent with continuing development. Thus,as the individual aged he or she would fall further behind developmentally. Since the time interval varied among the patients in the time of CNS treatment to the time of the psychological evaluation, analyses were done to determine whether time since treatment was associated with the degree of intellectual deficit. The patient's sibling score was used as an estimate of the patient's potential level of functioning. The patient sample was dichotomized on length

of time between treatment and time of testing and the two resulting groups were compared on the difference scores between each patient's score and his/her sibling's score. The longer interval group had the greater difference scores although none of these findings was statistically significant.

To test the hypothesis that younger children are the more impaired by CNS treatment, the patients in our sample were dichotomized into an older and a younger group (based on their age at the time of treatment) and difference scores between them and their siblings were derived in the same manner as described previously. The younger S's had significantly greater decrements in Full Scale and Verbal Scale IQ scores than the older group of patients. A non-significant trend in the same direction held for the performance scale IQ scores.

The age at treatment and the time interval since treatment were combined into one variable in order to maximize the variance contributed by these two theoretically related variables. (Age at the time of testing divided by the time since testing, yielded a score on the total percent of life lived since treatment). The patients were then dichotomized into those who lived either a greater or shorter percentage of their lives between treatment and testing; these two groups were compared as to the degree to which their scores differed from those of their siblings. The findings parallel those cited above, but the combined variable produced more highly significant differences than did the two separate variables.

To test the hypothesis that intellectual skills of brighter individuals tend to be more affected than those of average individuals by the diffuse brain damage likely to be produced by the CNS treatment, each patient was given an estimated pre-treatment IQ based on the IQ of the sibling. The sample was dichotomized as high average IQ. Difference scores were then computed between the patient and the sibling. The difference scores were greater for the patients with brighter siblings and most of the comparisons between groups were statistically significant. (There is very little evidence from a comparison sample that regression contributed in any major way to the apparent greater drop in IQ of patients with brighter siblings).

Other factors than brain damage could have contributed to the lowered intellectual functioning of the CNS treatment group. There are factors such as severe anxiety as a result of a life threatening illness, decreased environmental demands from adults as a concession to the health status of the child, and loss of learning opportunities. A sample of Ewing sarcoma patients and their siblings and a sample of cystic fibrosis patients and their siblings are currently being assessed.

Study II. Prospective study

A sample of 60 children with acute lymphocytic leukemia, who receive prophylactic CNS treatment, are being administered the same psychological evaluation described in the Post-treatment study. This assessment is made before CNS treatment and one year after treatment. This interval of one year should be

sufficient to allow for loss in learning abilities if such losses occur as the result of treatment. The sibling group from the Post-treatment study will be used as a control group for this study to partial out any practice effects that may contribute to the follow-up IQ scores among the patients.

Half of this sample will be between 3½ and 6 years of age and half will be between 6 and 16 years of age. This age distribution will provide a good basis for evaluating whether age at the time of CNS treatment is a factor in contributing to the degree of intellectual deficit that may occur.

A small group of children with optimal prognosis (between 4 and 7 years of age and with a white cell count of less than 10,000) also will be studied prospectively. Because of their unusually good prognosis, half of this group will receive intrathecal methotrexate without the CNS irradiation while the other half of this group will receive the complete CNS treatment. This sample will allow us to evaluate the effects of CNS methotrexate without irradiation.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00259-02 LDP

PERIOD COVERED

October 1, 1977 - September 30, 1978

TITLE OF PROJECT (80 characters or less)

Relations between Parent, Teacher, and Clinician Reports of Child Psychopathology

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Thomas M. Achenbach	Research Psychologist	LDP NIMH
Other:	Craig S. Edelbrock	Staff Fellow	LDP NIMH
	Faith Dickerson	Clinical Psychologist	Woodburn Center for Community Mental Health, Annandale Virginia

COOPERATING UNITS (if any)

Woodburn Center for Community Mental Health, Annandale, Va.

LAB/BRANCH Laboratory of Developmental Psychology

SECTION ---

INSTITUTE AND LOCATION

NIMH ADAMHA NIH Bethesda, Maryland

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.25	.10	.15

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purposes of the study are (1) to determine the relationships between parent, teacher, and clinician reports of the behavioral problems and competencies of children referred for outpatient mental health services; (2) to determine the relationship between children's presenting behavioral patterns as reported by these sources and the outcome of their clinic contacts. At intake, and again following termination with the clinic, parents and a clinician filled out the Child Behavior Checklist, while the child's teacher filled out a Teacher Report form. Child Behavior Profiles scored from these data will be compared to identify agreements and disagreements between parents, teachers, and the clinician. The relationship of outcome to intake Profile patterns, demographic variables, and other characteristics of the children will be assessed in order to identify predictors of poor outcome. If children who typically have poor outcomes can be identified, new treatment strategies will be developed to help them.

PHS-6040
(Rev. 10-76)

Project Description:

Little is known about the relationships between client characteristics and the effectiveness of existing treatments for child psychopathology. One of the purposes of the Child Behavior Profile (Project No. Z01 MH 00244-03 LDP) is to differentiate among children who differ in their responses to various treatment modalities. This study is designed to determine the degree of agreement and the nature of disagreements between Profiles scored from Child Behavior Checklists completed by children's parents, their teachers, and a clinician who did an intake and follow-up evaluation but was not involved in treatment. It is also designed to determine whether classification of children according to Profile patterns derived from reports by each type of informant separately and/or in composite is predictive of outcome in response to the child mental health services offered by a community mental health center.

In addition to the Behavior Profiles, the intake clinician obtained data on family composition, demographic characteristics, previous mental health contacts, degree of parent motivation for help, and the problems considered most serious by the parents. Six months after intake into the clinic, the intake clinician contacted the family to obtain Behavior Checklists describing the child's current status, plus data on changes in the problems considered most important by the parents, relevant changes in the family, the family's reactions to the services they have received, and possible needs for further services.

If children who have poor outcomes can be discriminated from those who have good outcomes on the basis of intake data, then new treatment strategies can be developed for helping children who are not likely to benefit from mental health services as currently offered. The data have been collected and are now being analyzed.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER ZO1 MH 00260-02 LDP

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)
Intellective Development in Normal and Deviant Infants

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Albert J. Caron, Ph.D.	Research Psychologist	LDP NIMH
	Rose F. Caron, Ph.D.	Adjunct Professor	Geo. Washington University
	Anita Sostek, Ph.D.	Pediatric Psychologist	Georgetown Univ. Hospital
	Patricia Quinn, M.D.	Instructor in Pediatrics	Georgetown Univ. Hospital
	Gordon Avery, M.D., Ph.D.	Director, Division of Neonatal Medicine	Children's Hosp.

COOPERATING UNITS (if any)
George Washington University
Georgetown University Hospital
Children's Hospital

LAB/BRANCH
Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.45	.85	.60

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The broad purpose of the project is to lay the basis for the development of a behavioral assay of intellective deficit in early infancy. Normal, premature, and Down's syndrome infants are being compared in terms of their ability to code relational features of stimuli under the assumption that the processing of patterned information is fundamental to intelligent behavior. Habituation-recovery procedures are being used to assess sensitivity to invariant spatial, temporal and social relationships between 48 and 78 weeks conceptional age. To evaluate predictive validity, performance in infancy will be related to cognitive and linguistic outcomes in childhood, and the extent of relationship will be compared with that of standard infant psychometric instruments.

PHS-6040
(Rev. 10-76)

Project Description:

Accumulating research evidence indicates that low birthweight and neonatal medical complications place an infant at risk for later intellectual and scholastic handicap. The high risk infant is of growing concern to public health, welfare and educational institutions, given (1) that some 9 per cent of live births in the U.S. are below 37 weeks gestational age and 5 per cent of term births are SGA, (2) that advances in neonatal care are steadily increasing the survival rate of the most vulnerable newborns (those under 1500 grams), and (3) that the incidence of various maternal factors correlated with high risk status (e.g., smoking, alcoholism, teenage pregnancy) appears to be on the rise.

It is important to be able to detect signs of future intellectual dysfunction as soon afterbirth as possible in infants at risk, in order to establish appropriate intervention programs to forestall the cumulative effects of such deficit as well as allow evaluation of the efficacy of the remedial programs. The objective of the present project is to develop a viable assay of intellective functioning early in life. The research focuses on an aspect of the infant's ability to abstract relational information. It is assumed that emerging intelligence is characterized by the capacity to isolate invariant properties of otherwise changing events and the extent to which such abstracted properties are structured rather than isolated and elemental in nature. It is hypothesized, therefore, that vulnerability to future mental deficit will manifest itself early in life as a difficulty in abstracting common spatial, temporal, and physico-structural relationships from the environment.

The project entails a number of related subgoals: (1) to refine existing knowledge about the ability of normal babies to form relational concepts and, concurrently, about the relative development of this capacity in at-risk and healthy infants; (2) to identify subcategories of vulnerable infants who are most at cognitive risk (CNS-involving trauma and post-natal respiratory distress); (3) to assess the extent to which sensitivity to relational attributes in infancy is associated with intellectual and linguistic outcomes in early childhood; (4) to determine whether high risk infants are impaired in socio-affective as well as cognitive development.

The past five months of this project have been devoted to developing tests of the infant's ability to form concepts about relational properties of stimuli. The relational properties include spatial and configural aspects of abstract visual patterns (e.g., above-below, inside-outside, etc., as well as structural features of meaningful social stimuli (faceness, facial expression, sex of face). The methodological technique employed is one in which the infant is habituated to a stimulus that varies from trial to trial in an irrelevant attribute but remains invariant in a target attribute. Following habituation the child is shown two novel stimuli, one altered on both the relevant and irrelevant dimensions, the other on only the irrelevant dimension. If the infant is able to abstract the relevant attribute during habituation, he should dishabituate only to the stimulus transformed on this attribute. The method has the added advantage of permitting assessment of

an individual infant's "sensitivity" to a given concept, in terms of the number of exemplars required to elicit abstraction of the invariant property.

It was found that normal, full-term 12-week-olds can abstract the constant shape of a figure viewed at varying rotations in the third dimension. Following habituation to a square presented at different slants, babies looked longer at an equal size trapezoid than at the same square when both were shown at a totally novel orientation. Control infants habituated to the trapezoid attended more to the square than the trapezoid. These findings imply, contrary to the long-held empiricist notion that depth perception is a product of locomotion in space, that prelocomotor infants can not only detect the true shape of objects seen at a slant but can also abstract this property as a constant element across multiple rotations. Also, 12-week-olds can discriminate two shapes shown at the same extreme slant.

A further study is currently underway in which normal 11- and 15-week-olds are being compared on their ability to abstract the spatial relationship of "above" (or "below"). While the older infants are performing somewhat better than the younger ones, findings thus far indicate a sex difference. Among older infants, 8 of 10 boys have successfully abstracted the relationship and only 2 of 10 girls; among younger infants the corresponding ratios are 6 of 10 and 1 of 6, respectively. If these percentages hold up with further testing, it could mean that the generally recognized differences between males and females on spatial tasks may have a non-cultural origin.

The infants' abilities in response to visual relational patterns such as symmetry and same-different, and abstract facial expressions (different facial photographs) are being tested. Initial findings again show sex differences. Whereas 6 of 6 girls successfully detected the difference in facial expressions, only 2 of 6 boys could do so. Although these findings require further replication, they appear to suggest that the social stereotype of greater sensitivity by girls to differences between social stimuli and by boys to differences between spatial abstract stimuli may have a basis in early infancy.

Testing of premature infants has begun. All prematures to be seen on this project will have been coded by hospital staff (and unknown to us) as likely or unlikely to have had CNS insult (intraventricular bleeding, seizures, prolonged assisted ventilation, etc.). They will also have been categorized as low birthweight (below 1800 grams) or moderate birthweight (1800-2500 grams).

Publications:

Caron, A. J., Caron, R. F., & Carlson, V. R. Do infants see objects or retinal images? Shape constancy revisited. Infant Behav. Develpm., 1978, in press.

Caron, A. J., & Caron, R. F. Effects of ecologically relevant manipulations on infant discrimination learning. Infant. Behav. Develpm., 1978, in press.

Caron, A. J., Friedman, S. L., Brackbill, Y., & Caron, R. F. Obstetric medication and visual processing in 4- and 5-month-old infants. Merrill-Palmer Quart. 24:111-128, 1978.

Caron, A. J., Caron, R. F., Michiello, M.D. Weist, S.J., & Friedman, S.L. Constraints on the use of the familiarization-novelty method in the assessment of infant discrimination. Child Develpm. 48:747-762, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00261-02 LDP

PERIOD COVERED
October 1, 1977 - September 30, 1978

TITLE OF PROJECT (80 characters or less)
Accuracy of Hierarchical Clustering Solutions in Multinormal Mixture Analyses

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Craig Edelbrock Staff Fellow LDP

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION
NIMH ADAMHA NIH Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.90	.45	.45

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords) A major statistical problem in mental health research has been how to translate subject's scores on a set of dimensions into a meaningful and reliable classification of individuals. This problem is particularly significant since decisions (e.g., assignment to different treatments) should be made on the basis of a subject's entire pattern of scores, rather than scores on a single dimension. The purpose of this research was to develop some new clustering algorithms that include corrections for unreliability, and to compare these algorithms using computer generated data sets. Parametric comparisons between hierarchical clustering algorithms were made, using several evaluative criteria including accuracy, precision, stress, and the cophenetic correlation coefficient. Algorithms using the product-moment correlation as the measure of similarity were superior to those using Euclidean distance, and those algorithms employing empirical Bayesian corrections for unreliability performed better than conventional algorithms.

PHS-6040
(Rev. 10-76)

Project Description:

A major statistical problem in mental health research has been how to translate subjects' scores on a set of single dimensions into a useful classification of individuals. This problem is particularly significant since decisions regarding diagnosis, treatment assignment, and delivery of services must be made about whole individuals, rather than their scores on rating scales, symptom checklists, or questionnaires. Cluster analysis is a family of statistical procedures that is becoming more commonly used in mental health research to produce classifications of individuals from a multivariate data set. Unfortunately, many clustering algorithms are available, different algorithms produce different results, and some algorithms are clearly unsuited for the type of data used in behavioral research. Hence, it is difficult to choose an algorithm for a clustering application, and to place confidence in the resulting typology.

This research compared several of the most widely used hierarchical clustering algorithms on their ability to retrieve the underlying data sets from multinormal mixtures. Five amalgamation rules were analyzed including (1) single or minimum linkage, (2) complete or maximum linkage, (3) average linkage (unweighted pairwise group mean average linkage), (4) centroid linkage (weighted pairwise linkage), and (5) Ward's error sum of squares technique. In addition a new algorithm, based on an empirical Bayesian modification of centroid linkage was developed and evaluated. With this algorithm, after each amalgamation, the resulting centroid is corrected for unreliability and expected regression to the centroid mean, thus producing a more reliable and precisely estimated clustering criterion. The purpose of this research was to supply empirical criteria for choosing a clustering algorithm and measure of similarity, and for deciding whether to standardize data before clustering.

The data sets were 10 multinormal mixtures, generated using Monte Carlo techniques. The number of underlying populations in each mixture ranged from 2 to 5, and the number of variables ranged from 7 to 22. Means for the variables were sampled from the range of 40 to 60, with a measurement error of $\pm$ .60 of the standard deviation of the variable. In addition, the ten data sets were prepared in either standardized or unstandardized form, resulting in a total of 20 data sets.

Accuracy of the clustering solution was defined as the agreement between the obtained clusters and the underlying populations in the mixtures. Accuracy was measured using the statistic kappa, a standardized measure of agreement between cross-classifications, and Rand's statistic, a measure based on pair-placements. Precision was defined as the degree to which the obtained clusters approximated the true population parameters of the mixture. Precision was measured by the error sum of squares function. In addition the cophenetic correlation and stress were calculated for each clustering solution.

Based on evaluations using 10 multinormal mixtures, the algorithms could be divided into two groups regarding their performance on these evaluative

criteria. The group having high accuracy and precision and low stress included average and centroid linkage algorithms using the product-moment correlation as measure of similarity, and Ward's minimum variance technique. In addition, the algorithm using the empirical Bayesian clustering strategy was better than conventional clustering algorithms. Complete and single linkage were consistently less accurate and precise than the other algorithms, regardless of the similarity measure used.

A manuscript summarizing this research is in preparation.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00263-02 LDP

PERIOD COVERED

October 1, 1977 - September 30, 1978

TITLE OF PROJECT (80 characters or less)

Behavioral Problems and Competencies of Clinic-referred and Nonreferred Children

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Thomas M. Achenbach	Research Psychologist	LDP	NIMH
Other:	Craig S. Edelbrock	Staff Fellow	LDP	NIMH

COOPERATING UNITS (if any)

25 Child Guidance Clinics and other mental health agencies

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION

NIMH ADAMHA NIH Bethesda, Md.

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.85	.25	.60

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In order to compare the behavioral problems and competencies reported for clinic-referred and nonreferred (normal) children, a survey of 1300 randomly selected parents in the Washington, D. C. area was conducted. Home interviewers administered the Child Behavior Checklist to parents of 50 boys and 50 girls at each age 4 through 16. The results are being compared with parental reports of problems and competencies for clinic-referred children matched to the nonreferred children for age, sex, socioeconomic status, and race. The data are expected to provide a unique epidemiological baseline for research on child psychopathology and the effectiveness of various treatments in bringing a child's behavior into the normal range for his age.

PHS-6040
(Rev. 10-76)

Project Description:

Although there have been a few normative studies of behavior problems in non-clinical groups of children and other studies of problems in clinical groups, there have been no systematic comparisons of problems and competencies reported for broad samples of clinic-referred and nonreferred children using the same instrument during the same period of time. Comparisons of this sort are badly needed in order to determine what kind of problems and competencies typically differentiate "normal" children from those who are recognized as being disturbed enough to warrant professional attention. Because types of problems and competencies are likely to differ with the age, sex, race, and socioeconomic status of the child, the study is designed to compare referred and nonreferred children matched for these variables at each chronological age from 4 through 16.

The data on the referred children have been collected with the Child Behavior Checklist administered in some 25 child mental health settings in the course of developing the Child Behavior Profile described in the Annual Report on Project No. Z01 MH 00244-03 LDP. The data on the nonreferred children were collected by selecting addresses at random within census tracts stratified so as to obtain a distribution similar to the distribution of clinic cases with respect to socioeconomic status and race. Letters describing the survey were mailed to the selected addresses and recipients were asked to return a postcard if they had no children aged 4 through 16 living with them. An interviewer then went to each address from which no postcard was returned. If a parent consented to be interviewed and if more than one child between the ages of 4 and 16 resided there, a random number table was used to select the child to be described on the Child Behavior Checklist, excluding any who had received mental health services within the previous year.

The scores obtained by the nonreferred boys on all behavior problem and social competence scales of the Child Behavior Profile have been found to differ at highly significant levels from those of clinic-referred boys within each age and socioeconomic group. Although lower socioeconomic parents reported more problems and fewer competencies on some scales than did upper socioeconomic parents, these differences were far smaller than the referred versus nonreferred differences across socioeconomic strata. Similar comparisons will be made on the Child Behavior Profile scales for girls when the Profiles for girls have been completed. Comparisons for both sexes on specific problems show highly significant differences between referred and nonreferred children for most problems, but the problems that best discriminate the groups vary greatly with the age and sex of the children. It is expected that these data will provide a unique epidemiological baseline for further research on child psychopathology and on the effectiveness of various treatment approaches in bringing a child's behavior into the normal range for his age.

Publications:

Achenbach, T.M. Developmental perspectives on psychopathology in children and adolescents. In Lamb, M. E. (Ed.): Social and personality development. New York, Holt, Rinehart, & Winston, 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00264-02 LDP

PERIOD COVERED
October 1, 1977 through September 30, 1978

TITLE OF PROJECT (80 characters or less)

Child Rearing Correlates of Young Children's Altruism

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Carolyn Zahn-Waxler	Research Psychologist	LDP	NIMH
Other:	Marian Radke-Yarrow	Research Psychologist	LDP	NIMH
	Robert King	Staff Psychiatrist	Children's Hospital	

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.25	.75	.50

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Maternal rearing behaviors were examined in relation to two kinds of moral behaviors manifested by children in the face of someone's emotional distress: (1) reparations to victims whose distress they had caused, and (2) altruistic responses to someone's distress that they had witnessed as bystanders were the focus of the study. Mothers provided systematic reporting of naturalistic and staged emotional incidents. Also, mother-child interaction was rated in periodic home visits by investigators. Data were collected over a nine month period. Mothers used a variety of emotional communications and teaching techniques when their children caused distress to someone. Mothers' use of emotionally administered explanations was positively associated with children's reparative behavior. The use of such communications was also significantly related to children's altruism when they were bystanders to another's distress. Empathic caregiving by mothers was positively related to children's altruism. Some differences in mothers' practices with boys and girls were evident (although no differences were apparent in the behaviors of boys and girls). Findings on relations between mother and child behaviors are considered in terms of theories regarding origins of altruism and conscience.

Project Description:

Young children are frequently exposed to other's emotions both as bystanders and also as causes of the emotions. Children's positive social initiations of comforting or aiding in these two kinds of eliciting circumstances provide evidence on what has traditionally been dealt with as altruism in the first instance, and "conscience", that is, reparations for distresses caused to others in the second instance. This investigation examines parental rearing practices with regard to these domains of moral behaviors in children.

The subjects are 16 mother-child pairs. The children are 1½ to 2½ years of age. The families are white, predominantly middle class.

Measures of mothers' rearing practices and children's prosocial behaviors were obtained over a period of nine months. The sources were observations in the home made at three week intervals and mothers' dictated reports of their children's and their own reactions to naturally occurring, and simulated incidents of emotional distress. (See project report ZO1 MH 00242 for procedures). Incidents included were those in which the child caused distress to someone and those in which the child witnessed distress as a bystander. Mothers' techniques in both kinds of circumstances were coded on content and affect. Children's altruistic and reparative responses were coded (e.g., help, comfort, sharing, sympathy). Percent agreements by coders of the mother and child behaviors were in the 80's.

Mothers used a variety of teaching and disciplinary techniques with their young children. In general, mothers provided more explicit teaching about feeling states of victims and about appropriate behaviors when their child caused the distress than when the child was a bystander witness. Emotionally charged reasoning about the consequences of the child's hurtful actions was a technique that was significantly related to the occurrence of reparative actions by the child toward the victim. Such emotional explanations with moralistic and judgmental overtones were also significantly related to children's altruistic acts in circumstances where they were bystanders to another's distress. Mothers' prohibitions about not hurting others when accompanied by explanations were positively related to children's reparation and altruism; unexplained verbal commands were negatively related to children's positive interventions. Maternal techniques in the child-caused distress situations tended to be the most predictive of children's moral behaviors. Mothers' empathic caretaking of the child and modeling of altruism (by comforting victims) were also linked with the child's prosocial responding. Maternal behavior with boys and girls suggested mothers' early anticipation that boys and girls are differentially sensitive to the emotions of others.

By the second year of life there were significant individual differences in children's interventions on behalf of another person.

This is a final report. A manuscript has been submitted for publication.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00265-02 LDP

PERIOD COVERED

October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Chronic Malnutrition and Child Behavior

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	David E. Barrett	Staff Fellow	LDP NIMH
	Marian Radke-Yarrow	Chief, LDP NIMH	LDP NIMH
OTHER:	Gale E. Inoff	Psychologist	LDP NIMH
	Staff		Institute of Nutrition of Central America and Panama; Univ. of California, San Diego

COOPERATING UNITS (if any)

Institute of Nutrition of Central America and Panama, Guatemala City, Guatemala; University of California, San Diego

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.75	1.00	.75

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This is an investigation of the effects of chronic malnutrition on the social, cognitive and affective development of children. The research encompasses two parallel studies, one in Guatemala, the other in San Diego, California. Six- to 10-year-old children identified as having had a history of malnutrition will be compared with non-malnourished peers on measures of functional competence. The identification of children on level of nutritional status will be based on previous nutritional history and present health and anthropometry. Behavioral assessments are made in individual and small group settings. Measures include (a) sensorimotor-cognitive variables: attentional properties, sensorimotor impulse control, reasoning and problem-solving; (b) social-emotional variables: motivational characteristics (persistence, exploration), work efficiency, moods and emotional responses, processing of social cues; and (c) social interaction variables: degree of involvement, positive and negative initiations, relationships established, emotional qualities of social exchanges.

PHS-6040
(Rev. 10-76)

Project Description

The research objective is to investigate the effects of a history of chronic undernutrition on the social, emotional, and cognitive development of children, i.e., on the functional competence of children.

Evidence on the behavioral characteristics of malnourished organisms comes primarily from experimental animal research. With some regularity and replication across species and studies, malnourished animals and those with histories of malnutrition have been found to behave differently from their nourished peers. Passivity, apathy, and inability to sustain attention are widely reported. Malnourished animals compared with their healthy peers show reduced exploration, more fearful avoidance of new stimuli, nonpurposive behavior, excitability, and failure to respond normally to social initiations from other animals. In studies of malnourished animal infants and their mothers, disturbed interactions are reported.

In general, the behavioral consequences of early chronic malnutrition on the functioning of children have not been well-investigated. Most studies of malnourished children have focused on intellective capacities. Little research has been directed to the effects of nutritional deficit on the child's social and emotional functioning -- capacities for learning, for dealing with environmental demands, and for functioning in the social order. Yet, the behavioral impairments identified in the animal research, and reported descriptively in work with humans, strongly suggest functional problems. Failure to sustain attention, lack of impulse control, aimless activity, and heightened emotionality, if characteristic of children, might well lead to learning disabilities, impaired coping with environmental demands, disordered emotional responding, and incompetent day-to-day functioning.

The primary hypotheses in this study are that the chronically malnourished child is characterized by attentional disorders, general apathy, and lessened control of impulses and emotional responses. These properties, it is hypothesized, have a progressive and cumulative effect on the child's behavior and on the reactions of others to the child. The most direct consequences of attentional deficit and apathy would be expected to be impaired information processing capacity, heightened emotionality, and poor impulse control. Given the hypothesized dysfunctions, a number of predictions can be made to the child's motivation, efficiency, and social functioning: namely, (a) that low exploration and innovation, low persistence, and erratic and emotional behavior become patterned ways of responding; (b) that, because of ineffective attending, the child is not alert to social cues from others and hence is less competent in social interaction; (c) that, as problems of coping lead to difficulties and failures, negative attitudes and affect toward the self develop; (d) that a child with the hypothesized characteristics elicits more negative responses from others.

The present research was originally designed as a nutritional intervention study to be carried out in India. Because of delays and complications in

collaborative arrangements, the study was reformulated and new sites and populations chosen. The research now encompasses two studies: one to be carried out in Guatemala, the other in San Diego, California.

In the study to be carried out in Guatemala, a sample of 200 6- to 10-year-old children who have been participants in INCAP's longitudinal research (begun in 1969) will be studied. Their selection is based on (a) their nutritional history (i.e., the best assessment of year to year food intake: the kind and amount of supplement they consumed in the INCAP program and the home food intake, both expressed in terms of intake of specific nutrients) and (b) current anthropometric measures (a lower cut-off percentile of height-for-age within the Guatemalan population, and, in addition, measures of weight and skinfold). Children who on these criteria appear to have had histories of inadequate nutritional intake will be compared with children who have had more nearly adequate histories of nutritional intake.

In the course of this study, food is not an experimental variable. All families in the villages in which the study is carried out will be provided the same family food supplement of enriched flour.

In the study to be carried out in San Diego, subjects will be 6- to 10-year-olds. Selection of the sample is on the basis of height-for-age. The primary sample is composed of children who are at or below the 10th percentile in height-for-age. An additional sample is at or above the 50th percentile. Additional criteria involve anthropometry of child and family, health and nutritional history of mother during pregnancy, health and nutritional history of the child, and the child's current health status. A medical clinical judgment based on the combined criteria will be made to assign the child to a group: small-for-age due to a history of undernutrition; small-for-age due to genetic reasons: or average height-for-age, adequately nourished.

The composition of the sample will be Caucasion, Black, and Mexican-American. The projected sample is 120 children.

Since malnutrition occurs as part of a configuration of other difficult conditions of living, in both studies information will be obtained on non-nutritional factors such as family living conditions, income, education, and family size.

In both studies, psychological assessments will be made (a) in individual sessions and (b) in small groups (6 to 8 children per group). The individual and group assessments will be integrated into three to four half-day sessions, distributed over a period of two to three weeks. Individual assessments will be made on (a) sensorimotor-cognitive variables: attention and information processing, sensorimotor impulse control, reasoning and problem solving, and (b) social-emotional variables: motivational characteristics, work efficiency, emotional qualities, attention and information processing of social cues. In the child groups, activities will involve free play, group projects, shared commodities, and group problem-solving. These are activities in which inter-

action with others (peers and adults) can be measured. The activities are programmed to control a variety of dimensions of social experiences. Variables will include social involvement, characteristics of social response and interaction, emotional characteristics, and responses elicited from others.

The preliminaries of instrument development are underway and case finding procedures are being initiated. Field work will take place during the coming year.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00266-02 LDP

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)
Newborn Interaction with Mothers and Nonmothers

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Jacob L. Gewirtz, Ph.D.	Research Psychologist LDP NIMH
Other:	Robert L. Manniello, M.D.	Rancho Palos Verdes, California
	Albert R. Hollenbeck, Ph.D.	Staff Fellow LDP NIMH

COOPERATING UNITS (if any)
Alexandria Hospital, Alexandria, Virginia
Holy Cross Hospital, Silver Spring, Maryland

LAB/BRANCH
Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION
NIMH ADAMHA NIH Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.05	.70	1.35

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords) This investigation has four aims: (1) To investigate the effects of 2 types of contact (skin-to-skin vs. cradling) and 2 durations (15 vs. 60 min.) of contact between mother and newborn in the first post-partum hour on the mother's attachment to her infant, their interaction, and the infant's behavioral development, at 2 and 28 days of age; (2) To investigate the effects of 2 durations (5 vs. 60 min.) of contact between father and his C-Section newborn in the first 1 1/2 hours post partum on the father's attachment to his infant, their interaction, mother's interaction with infant, and infant's behavioral development, at 3 and 28 days of age; (3) To determine if a differential attachment to a newborn infant at 3 days can be fostered in nonmothers (nursing students) by a program of intense interaction with that newborn during its post-partum hospital stay; (4) To determine if, at 3 days, the quality and quantity of interaction of a newborn with its mother, and it behavioral development (as indexed by the Brazelton Neonatal Scale), can be fostered by a concurrent program of intense interaction between that newborn and a nonmother interactor. Twenty second-year nursing students, 80 full-term newborn infants and their mothers, and 50 C-section-delivered newborns and their fathers and mothers, will serve as subjects.

Project Description:

The aims of this project are:

1. To investigate the effects of different types (skin-to-skin, cradling) and durations (15 vs. 60 minutes) of contact between the mother and her newborn in the first post-partum hour on the mother's attachment to her infant, their interaction, and the infant's behavioral development, at 3 and 28 days of age;

2. To investigate the effects of two durations (5 vs. 60 minutes) of contact between the father and his Caesarian-section-delivered newborn in the first 1 1/2 hours post partum on the father's attachment to his infant, their interaction, the mother's attachment and interaction with the infant, and the infant's behavioral development, at 4 and 28 days of age.

3. To determine if a differental attachment to a newborn infant can be fostered in nonmothers (nursing students) by a program of intense interaction, beginning at birth, with that newborn during its post-partum hospital stay; and

4. To determine if, at 3 days, the quality and quantity of interaction of a newborn with its mother and its behavioral development can be fostered by a concurrent program of intense interaction between that newborn and a nonmother (nursing student) interactor.

The immediate post-partum mother-infant and father-infant contact manipulations are relevant to issues in the development of parental "attachment-bonding" to infants. Recent work has drawn attention to the fact that skin-to-skin post-partum contact between neonate and mother in the first hour (deChateau, 1976; Hales et al, 1977), and contact opportunity during the first three days (Klaus, Jerauld, Kreger, McAlpine, Steffa, & Kennell, 1972), could result in affectionate maternal behaviors denoting attachment to the infant, both in the neonatal and subsequent periods. The manipulations employed in this investigation have the primary purpose of replicating and extending, in a controlled setting, studies of the possible effects of immediate post-partum mother-infant contact on the development of maternal behaviors denoting attachment to the infant, and of identifying some features of parent- and nonmother-infant interchange that can account for the development of parent and nonmother attachment to the infant.

Married mothers of full-term infants, vaginally delivered without complication under epidural anesthesis, and their normal infants serve as subjects under Aims 1, 3 and 4. Mothers receive instructions to guide their interactions with their newborns in the recovery room (Aim 1) or are approached then to permit interaction with, and observation of, their infants in the nursing-student study (Aims 3 and 4). Infants delivered without complication via routine C-Section procedures, and their fathers and mothers, are the subjects under Aim 2. Fathers are given instructions to guide their interaction with their newborns right after delivery. An area is set aside in the special-care nursery. Under all aims, infants are assessed as normal full-

term newborns with Apgar scores >8 at five minutes of age. During the first post-partum hour, infant temperatures are taken initially and at 15-minute intervals while neonates are with mothers. In no case does a mother or infant spend less time in the recovery area than is usual at the hospitals employed (60 minutes for mothers, 15 minutes for neonates). Assignment of parent-infant pairs or infants to treatment conditions is done at random.

For Aims 3 and 4, some 20 second-year nursing students are the subjects. Aim 4 is stressed as the focus of the study; i.e., whether or not intensive interaction of caregivers with newborns ("primary" infants) could foster the development of newborn social behavior. Under Aim 3, each student is assigned a primary (experimental) and a secondary (control) infant for the 2-3 days of their service. The intensive interaction with her primary infant, that constitutes the treatment condition under Aim 3, is governed by a script that calls for repeated tactile and auditory stimulations, and repeated assessments of consolability, cuddliness, and visual fixation of the infant (according to the Brazelton Neonatal Behavioral Assessment Scale). In addition, the student keeps a running diary of the primary infant's states, responses to stimuli, and behavioral assessments.

Under Aim 1, mother-infant contact in the recovery room during the first post-partum hour occurs under one of four conditions: 1) 60-minute ventral-ventral skin-to-skin contact, where the naked newborn is placed on the mother's naked abdomen, is supported by her in an encompassing manner, and is covered with a blanket (to minimize heat loss); 2) 15-minute skin-to-skin contact as in (1); 3) 60-minute cradling, where the newborn, wrapped in a blanket, is nested in the crook of its mother's elbow; and 4) 15-minute cradling, where, as in (3), the wrapped neonate is nested in the crook of its mother's elbow.

Observations of the newborn with nursing student, mother, or father are made, using the following behavior categories: For mother, father, or nursing-student--reposition, smile, enface looking, body looking, talk, vocal non-language sound, making visual spectacle, finger contact, palm contact, rock, hold, kiss, grimace, feed, and imitate; For infant--grimace, smile, enface looking, eyes open, nonvocal sound, vocal sound, mouthing, fuss-cry and motor movement. In addition, responses of mother, father, nursing student, or child contingent on responses of the other are scored.

Under Aim 1, an observation of the initial interchange between mother and neonate is made in the recovery area. In addition, two 15-minute observations of mother and infant during a feeding and between feedings are made at 48 hours and at 28 days after birth. Also, an assessment using the Brazelton Neonatal Behavioral Assessment Scale is made at those two time points, and a diary of mother-infant interaction is collected and ratings of their behavior characteristics made at 28 days.

Under Aim 2, an observation of the initial interchange between father and C-section-delivered neonate is made right after delivery in the special-care nursery. In addition, one feed and one nonfeed 15-minute observation of

mother and infant are made at 72 hours and at 28 days after birth, and a 15-minute nonfeed observation of father and infant is made at 96 hours and at 28 days after birth. Also, the infant's behaviors (Brazelton Scale) are assessed at 48 hours and 28 days. Finally, a diary for father-infant and mother-infant interaction is collected and ratings of their behavior characteristics are made at 28 days.

Under Aims 3 and 4, two daily 15-minute observations of the interchange between nursing student and primary (experimental) infant, between nursing student and secondary (control) infant, and between those infants and their mothers at 48 hours are made. In addition, under Aim 4, an assessment of both experimental and control infants at 48 hours using the Brazelton Scale is made.

To date, partial and complete series of observations and tests have been done, as follows: for Aim 1, a total of 38 cases under the four recovery-room presentation-of-infant-to-mother conditions; for Aim 2, a total of 36 cases under the two special-care nursery father-infant-interaction duration conditions; and for Aims 3 and 4, some 20 cases. Sampling is continuing under Aims 1 and 2.

Publications

None.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00267-02 LDP

PERIOD COVERED

October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Studies on the Impact of Television on Families with Young Infants

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Albert R. Hollenbeck Staff Fellow LDP NIMH

Other: None

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Developmental Psychology, NIMH

SECTION

INSTITUTE AND LOCATION

NIMH ADAMHA NIH Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.10	.05	.05

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The present project was designed to assess the influence on infants of exposure to television in the home. 72 6-month-old infants were exposed on 4 consecutive days to a 16-minute session in which 4-minute segments contained either the sound only, the picture only, the picture plus sound of television; or an unpatterned control stimulus. Visual behavior data indicated that infants looked more at the picture-plus-sound of television than at the other presentations. Also, infants looked more at the picture only presentation than at the sound only presentation. Vocal behavior data indicated that infants spent the least amount of time vocalizing during the picture-plus-sound of television than during the other three presentations. Also, infants cried more often and for longer duration during the picture-only presentation than during the sound only or the picture-plus-sound presentations. These results indicate that: (1) infants respond to television programs in the home environment, and (2) infants respond differentially to visual and auditory forms of stimulation provided by television.

PHS-6040
(Rev. 10-76)

Project Number Z01 MH 00267-02 LDP

Project Description:

The aim of this project was to provide a first assessment of the potential significance of television for infants in the naturalistic setting of the home. The influence of television on infants' visual and vocal behavior was studied. In addition, data were collected on television viewing patterns of families and on mother-infant relations using the Home Observation for Measurement of the Environment Inventory (HOME).

To provide a first assessment of television influences on infants in the naturalistic setting of the home, visual and vocal behaviors of 72 infants six months of age were recorded during four days of exposure to various components of broadcast television programs: sound only, picture only, sound plus picture, or a control stimulus of unpatterned sound plus picture. Infants looked longer at the television set during the sound-plus-picture condition than during the picture-only condition, and they looked longer during both of these patterned picture conditions than during both of the other conditions that lacked a patterned picture. Also, infants vocalized more during the picture-only condition than during the sound-only condition. These findings demonstrate that infants attend to the naturalistic presentation of television stimulation, and they respond differentially to the visual and auditory components of television. Results are discussed in terms of the question of the long-term influence on infants' social responsiveness of substantial levels of exposure to the non-contingent, quasi-social stimulation provided by television.

Data on this project was collected prior to appointment in the Laboratory. Time in the Laboratory was devoted to analytic work on these studies. This is a final report.

Publications:

Hollenbeck, A. R. Television viewing patterns of families with young infants. J. Soc. Psychol., in press, 1978.

Hollenbeck, A. R. Early infant home environments: Validation of the Home Observation for Measurement of the Environment Inventory. Devel. Psychol., in press, 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00269-01 LDP

PERIOD COVERED
October 1, 1977 - September 30, 1978

TITLE OF PROJECT (80 characters or less)

Assessment of the Outcome of Child Psychiatric Treatment

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P. I:	Thomas M. Achenbach	Research Psychologist	LDP NIMH
Other:	Craig S. Edelbrock	Staff Fellow	LDP NIMH
	David Caine	Clinical Psychologist	Clifford Beers Clinic, New Haven Conn.
	Rosemary Downs	Research Psychologist	" " "

COOPERATING UNITS (if any)

Clifford Beers Guidance Clinic, New Haven, Connecticut

LAB/BRANCH
Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION
NIMH ADAMHA NIH Bethesda, Maryland

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.45	.30	.15

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose is to assess relations between the patterns of problems and competencies displayed by children referred for guidance clinic treatment and the outcome of two widely offered treatment modalities, namely, individual psychotherapy and conjoint family therapy. The subjects are applicants to a guidance clinic who manifest behavior patterns corresponding to several pre-selected types and whose therapists judge individual psychotherapy and conjoint family therapy to be equally good treatment modalities for them. If the child and family consent, the therapist administers either psychotherapy or family therapy, as determined by the research design. The course and length of therapy depend upon the child's progress and the therapist's judgment, but an independent evaluator obtains ratings from the parents and the child's teacher at the beginning of treatment and again at 6 and 12 months after intake.

PHS-6040
(Rev. 10-76)

Project Description:

Despite the large number of children in need of mental health services, there have been very few controlled studies of the effectiveness of such services. Even the best of the few existing studies suffer from a fundamental defect: They have assessed the effectiveness of treatment indiscriminately across heterogeneous groups of children. Despite the fact that no single approach to treatment is likely to be uniformly optimal with all kinds of children from all kinds of backgrounds manifesting all kinds of problems, these studies have not been designed to determine what kind of treatment is most beneficial for which children.

The purpose of the present study is to perform a controlled evaluation of the effectiveness of different outpatient treatment modalities with children who are grouped according to demographic characteristics (age, race, sex, socioeconomic status) and according to patterns of behavioral problems and competencies scored on the Child Behavior Profile. The Profile has been developed by Dr. Achenbach as a standardized instrument for describing children's problems and competencies, for identifying overall behavior patterns, and for measuring behavioral change from the Child Behavior Checklist. Cluster analysis of Profile patterns has made it possible to identify a limited number of "types" that characterize the vast majority of children seen in outpatient psychiatric facilities. This typology of Profile patterns provides a basis for classifying children in order to determine which ones benefit most from which type of treatment. In our study, applicants to Clifford Beers Guidance Clinic who are matched with respect to age, sex, socioeconomic status, race, and Child Behavior Profile type receive one of two treatment conditions: Individual psychotherapy or conjoint family therapy. The focus is not on which therapy is the better of the two but on differences in outcomes for different types of children who have received the two different types of treatment. Thus, several highly informative findings are possible. For example, it might be found that certain types of children do well under one treatment and poorly under the other. This would provide a basis for more precise prescription of treatment based on child characteristics. Another possible finding is that certain types of children do well under both treatments and other types do poorly under both treatments. This would indicate the need for trying a wider range of treatment modalities with the latter group or of developing new approaches for them if no existing approach is found to be effective. Whatever the outcome for each group, the fact that children who are similar with respect to Profile type, age, sex, race, and socioeconomic status will have received different treatments according to a systematic research design will permit a far stronger basis for inferring the effects of each treatment with each type of child than is possible without controlled assignment to treatment conditions.

The initial phase of the study includes only boys aged 6 through 11 having any one of four behavior Profile types we have identified for this group. The reason for beginning with this group is that they are by far the most

numerous clients of child guidance clinics. Because the number of participating therapists is limited, and because each therapist has continuing responsibility for the children who become part of the study, the chances of obtaining at least 10 subjects in each of the 4 (Profile types) x 2 (treatment types) = 8 cells of the research design are best with this group. However, if the research design is completed with this group well within the three years planned for the study, then similar procedures will be followed with girls and/or with boys aged 12 through 16.

Boys aged 6 through 11 who have one of the four target Behavior Profile types and whose therapist judges individual psychotherapy and conjoint family therapy to be equally appropriate for them are assigned to the two treatment conditions in such a way as to produce distributions that are as similar as possible with respect to age, race, and socioeconomic status. This is done by having an independent evaluator score the child's intake Profile after the therapist's clinical evaluation but before the therapist meets again with the family to discuss treatment. If the Profile corresponds to one of the four target types, the independent evaluator determines which of the two treatments should be provided as follows: For the first 10 cases having each Profile type, the evaluator alternates treatment assignments so that the first case having either of two Profile types receives individual therapy, whereas the first case having either of the other two Profile types receives conjoint therapy. Thereafter, the second case of each type receives the treatment that the first case did not receive, and the alternation of assignments to treatment continues until five cases having a particular Profile type have been assigned to each treatment. Because she maintains a record of the age, race, and socio-economic status of each case, the independent evaluator will be able to identify and correct any important differences in the distribution of these variables between cells of the research design.

If a family consents to participate in the study, the data from the Child Behavior Checklist they filled out as a routine part of Beers' clinical evaluation, plus a Teacher's Report obtained with the parents' permission, and the therapist's ratings on family functioning provide the baseline against which behavioral change is measured. Since all the participating therapists are skilled in both types of therapy to be offered, the same therapist will treat the child regardless of which therapy is selected according to the treatment design. The length of treatment is determined by the therapist's clinical judgment and the family's motivation, rather than by the research design. Whenever termination occurs, the therapist rates the child and family on various aspects of functioning.

The major focus of the statistical evaluation will be on changes in the behavioral problems and competencies reported by parents, therapists, and teachers from intake until termination and until the 6- and 12-month follow-ups for children of each Profile type receiving each type of treatment. One dimension of analysis will be a comparison of outcomes for children having a particular Profile type but receiving the two different treatment modalities. This will be done in order to determine whether one type of treatment

has been more successful than the other type of treatment with children having a particular Profile type. A second dimension of analysis will be a comparison of children having different Profile types in order to determine whether Profile pattern is consistently related to outcome, regardless of type of treatment. The relationship of the therapists' intake ratings to the outcome measures will also be assessed in order to determine whether they predict outcome independently of or in conjunction with Profile type and treatment modality.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-MH 00270-01 LDP

PERIOD COVERED

October 1, 1977 - September 30, 1978

TITLE OF PROJECT (80 characters or less)

Identification and Validation of Syndromes of Child Psychopathology

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Craig S. Edelbrock	Staff Fellow	LDP NIMH
OTHER:	Thomas M. Achenbach	Research Psychologist	LDP NIMH

COOPERATING UNITS (if any)

25 Child Guidance Clinics and other Mental Health Agencies

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.45	.25	.20

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In order to identify and validate syndromes of child psychopathology, we have conducted a literature review of all efforts to derive such syndromes empirically, have made cross comparisons among studies to identify replicated syndromes, and are performing cluster analyses of Child Behavior Profiles in multiple clinical samples. Clusters found to replicate across samples are used to classify children in order to identify demographic, diagnostic, adaptive, and prognostic correlates of the syndromes thus represented

PHS-6040
(Rev. 10-76)

Project Description:

The lack of an accepted taxonomy for child psychopathology has stimulated many efforts to identify syndromes of behavior problems through multivariate statistical techniques. However, the diversity of subject samples, raters, data collection instruments, techniques of analysis, and methodological flaws made it necessary to do a systematic review and cross-comparison of findings in order to determine whether there is consistent evidence for any syndrome, despite the methodological differences among studies. We found that a distinction between broad-band and narrow-band syndromes seemed warranted because many syndromes derived from first-order factor analyses could be subsumed by a few second-order factors. Broad-band "undercontrolled" and "overcontrolled" syndromes and narrow-band aggressive, delinquent, hyperactive, schizoid, anxious, depressed, somatic, and withdrawn syndromes were found in diverse samples of disturbed children. Two other broad-band and six other narrow-band syndromes were found in a few studies. Test-retest reliabilities and stabilities of syndrome scores were more adequate than interrater reliabilities, which were inversely related to the degree of disparity between types of raters and situations in which they saw subjects. Cross-instrument and cross-population consistencies corroborated some empirically derived syndromes, but the lack of independent criteria for categorizing disturbed children makes it difficult to establish criterion-referenced validity.

Because most efforts to derive syndromes empirically have not been extended to categorization of individual children and the few previous efforts have been limited primarily to the broad-band undercontrolled versus overcontrolled dichotomy, we are applying cluster analysis to Child Behavior Profiles in order to obtain a more differentiated taxonomy. Profile patterns that are replicated through cluster analyses of multiple clinical samples are used to classify children. Differences among the children in demographic characteristics, social competencies, psychiatric diagnoses, and the outcomes of clinical contacts are then assessed in order to determine the correlates of the cluster-based taxonomy.

Publications:

Achenbach, T. M.: Psychopathology of childhood: Research problems and issues. J. Consult. Clin. Psychol., in press.

Achenbach, T. M., and Edelbrock, C. S.: The classification of child psychopathology: A review and analysis of empirical efforts. Psychol. Bull., in press.

Achenbach, T. M.: Assessment of childhood behavior disorders. In Woody, R.: Encyclopedia of Clinical Assessment. San Francisco, Jossey-Bass, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER 02230 Z01 MH ~~00271~~-01 LDP

PERIOD COVERED

October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Conditions Influencing Interaction between Parents, Nonparents, and Neonates

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Jacob L. Gewirtz, Ph.D.	Research Psychologist	LDP NIMH
Other:	Albert R. Hollenbeck, Ph.D.	Staff Fellow	LDP NIMH

COOPERATING UNITS (if any)

Alexandria Hospital, Alexandria, Virginia
Columbia Hospital for Women, Washington, D.C.

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION

NIMH ADAMHA NIH Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.50	.85	.65

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords) This study focuses on the influence of infant characteristics on parent behavior. Effects on parents' behavior of the attribution to the infant of particular appearance or behavior characteristics are examined. First, parents and nonparents are asked to provide infants with abstract commodities: "warmth," "love," "comfort," "reassurance," and "security." For mothers, fathers, and nonparents, analyses are made of the interaction patterns to which each abstract commodity reduces, and how those patterns relate to situational and demographic variables. Second, similar observations and comparisons will be made of nonparents when they interact with infants arbitrarily labeled male or female. Third, following a standard exam of the infant, the investigator makes a statement to the parent, emphasizing a transient infant characteristic. In a subsequent observation, the pattern of parental responses in interaction with the infant (comprised of hugs, smiles, verbalizations, kisses, squeezes) is assessed. This is compared to patterns that follow comparable statements about an unfamiliar infant and a neutral statement about their infant's characteristics, and can be related to (a) situational variables (e.g., presence of the other parent or a stranger) and (b) demographic variables (e.g., infant gender, birth order).

Project Description:

A unidirectional model of the effects of parental behavior on the child has come to be seen as too simple to explain the complex interactions that transpire between child and parent. There has been an increasing emphasis on the role of the child in influencing the behavior of its parent or caregiver. In this study, conditions of contact between infant and mother in the first post-partum days are explored systematically to understand how the infant's characteristics can influence the behavior of parents and others. The working assumption is that an instructional set or disposition given to an adult about particular infant characteristics is systematically equivalent to the set or disposition the adult would acquire from the characteristic itself, in its potential for inflecting the adult's behavior toward the infant. Adults will be instructed on how to interact with an infant, or a particular physical characteristic (e.g., gender) will be attributed to the infant and, in a standard situation, the interaction between infant and adult that results is observed.

Primiparous mothers and their newborn infants are observed in the mother's hospital room in the first post-partum days. Other adults are observed with the newborns in a separate room. One videotaped observation of each adult-neonate pair is made, lasting approximately 15 minutes. The coding system used is designed to be descriptive of individual adult and infant behaviors. Adult dimensions include categories for caregiving, holding, tactile stimulation, looking, facial expression, and vocal stimulation. Infant dimensions include categories for visual behavior, facial expression, vocal behavior, and motoric behavior. An electronic device is used to obtain frequencies and durations of behaviors. Observers are blind to the instructional predisposition that is given to adults by an experimenter.

Changes in adult-infant interaction will be evaluated in relation to treatment manipulations under three related yet operationally-independent themes. The three experimental adult-behavior induction conditions stand as independent studies that utilize a common control group. The first condition is to investigate and compare adult response patterns when adults are instructed on how to interact with infants. Nurturance has been an important variable in descriptions of infant development, yet terms such as "love" and "comfort" are at best idiosyncratic to individual studies and at worst ambiguous as descriptors of adult behavior. We intend to examine nurturance in a more direct fashion. Thus, the adult is asked to show "love" and "comfort" to the infant. As a within-subject control, the adult is given a final instructional set simply "to get to know your infant." Each instructional set is given to the mother with her own child, the mother with a stranger child, a nonmother stranger, and the father with his own and a stranger child. The order of presentation of sets is counterbalanced in a Latin-square design. From these observations we hope to develop a taxonomy of adult behaviors to infants that define abstract terms like "love" and "comfort," as well as to compare behavior patterns within and between classes of adults.

A hypothesis about adult behavior that persists in the child-rearing

literature is that adults respond differentially to children depending on the child's sex, and that this differential treatment, in turn, explains later differences in behavior of boys and girls. The aim of our second condition is to compare the response patterns of new parents and nonparents to infants, when those infants are arbitrarily labeled male or female. Again, mothers, fathers, and stranger nonmothers will be told that a stranger infant with whom they are to interact is a boy or a girl--regardless of its true sex. Each infant will be counterbalanced to serve both as a boy and as a girl with different adults. Specific comparisons will be made of individual adult behavior patterns as detailed previously. Furthermore, these comparisons can be made in terms of the true sex of the stranger infant.

Social situations may also be influenced by implicit messages communicated to parents by persons having contact with their baby. The aim of the third condition is to investigate how adult-infant interaction can be influenced by an attribution to the infant of particular behavioral characteristics. This will be accomplished following a nominal examination of the infant at which the adult subject is not present. A staff member will remark to the adult that, during the examination, the infant was (a) "active," or (b) "happy," or (c) the adult is simply given the opportunity to interact with the baby. This manipulation series again uses a within-subject control procedure. The mother, the father, a stranger mother, and/or a stranger nonmother will be handed the infant and asked to "take charge of the baby while the observer watches the baby." The baby is handed to the adult after the description. From these operations we hope to describe how infant-behavior characteristics--effected by an instructional set--directly influence adult behavior patterns, both within and between adult subjects.

The final condition is a nonexperimental control condition where induction of adult behavior is minimized. Mothers and fathers (with their own neonates), and a stranger mother and a stranger nonmother (with infants of other parents) will be told that they are being given the opportunity to interact with the infant. This condition is identical to the previous within-subject control groups where adults are asked to get to know the infant. The purpose of this condition is to provide direct comparisons between each of the previously-listed manipulations, including within-subject control conditions, during a prolonged observation where adult-infant interaction behaviors are not manipulated intentionally.

Few data are available on the processes whereby infant appearance and behavior, and expectations about infant behaviors, influence adult behaviors to infants. This research is a modest initial attempt at examining several such processes.

Work on data collection and analysis is underway.

Publications

None.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER 02132 Z01 MH ~~00272~~-01 LDP

PERIOD COVERED
October 1, 1977 through September 30, 1978

TITLE OF PROJECT (80 characters or less)
Children's Misdeeds, Parental Disciplinary Practices and Children's Learning

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Carolyn Zahn-Waxler Research Psychologist LDP NIMH

COOPERATING UNITS (if any)
None

LAB/BRANCH
Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.65	.20	.45

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The aim of this research is to examine the roles of children and parents in influencing the kinds of discipline used and the outcomes of parental discipline. Do given types of misdeameanors by children tend to evoke characteristic forms of discipline from parents? Do given forms of discipline used by parents have predictable outcomes for children's behavior? The sample is 24 middle class mothers and their children who are in the second and third years of life. The data are reports by mothers trained in observation techniques. Children's misdeeds, mothers' reactions to these misdeeds and children's behaviors following discipline were investigated. Categories of misdeeds were harm to people, harm to property or objects, and "lapses" in self-control (e.g., temper tantrums). Forms of discipline included reasoning, verbal prohibitions, physical punishment, love withdrawal, guidance in appropriate behavior. Children's compliance and noncompliance to the discipline were coded. Analyses focus on the possible bidirectional effects of children's transgressions and parents' disciplinary practices in the resolution of conflict episodes.

PHS-6040
(Rev. 10-76)

Project Description:

Theories of socialization most often have been formulated in terms of a unidirectional model: The influences on children's behavior of different parental rearing and disciplinary practices (e.g., physical punishments, reasoning, withholding of resources) are examined. More recently, increased attention has been given to the role of child characteristics in determining parents' reactions to their children. The aim of this research is to examine the roles of parents and children in influencing the outcome of episodes involving parental discipline. Two questions are addressed: Do given types of misdemeanors by children tend to provoke characteristic forms of discipline from parents? Do given forms of discipline used by parents have predictable outcomes for children's behavior?

The sample is 24 middle class mothers and their children ages 1 to 2½ years.

The data are mothers' dictated observations of specific incidents of misdeeds by their children, disciplinary reactions to these misdeeds and the children's behaviors following discipline. Observations were made over a nine month period. (For descriptions of the training and reporting procedures for mothers see Project Z01 MH 00242). Incidents were coded for type of misdeed, type of discipline by mother, and child's response to discipline.

Twenty-one of the twenty-four children were reported to have transgressed in each of the three categories of misdeeds and to have been disciplined. These 21 mother-child pairs were the basis for analyses. Analyses of variance are used to examine the possible effects of both children and parents in determining the outcome in these conflict episodes.

Publications

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do **NOT** use this space)

U.S. DEPARTMENT OF
HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER 02133
Z01 MH ~~00273~~-01 LDP

PERIOD COVERED
October 1, 1977 through September 30, 1978

TITLE OF PROJECT (80 characters or less)
Stability and Alterability in Children's Responsiveness to Others' Emotions

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Carolyn Zahn-Waxler	Research Psychologist	LDP	NIMH
Other:	Marian Radke-Yarrow	Research Psychologist	LDP	NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION
NIMH ADAMHA NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.50	.20	.30

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The development and socialization of emotional responsiveness of young children to the feelings and emotions of others are considered in two research questions: (1) How predictive of later affective behavior are behaviors early in life? (2) Can the young child's emotional responsiveness be predictively altered by planned rearing interventions? Investigation of the first question involves a four year follow-up of children who were assessed in the second year of life and who then showed relatively stable individual patterns of reactions to others' emotions, e.g., sympathy, avoidance, indifference, anger. Data gathered on the follow-up parallel those of the original study: the mother's dictated observations of the child's responses to incidents of affective expression in another person. The second research question involves planned interventions by mothers to facilitate empathic sensitivity and altruistic responding in their children. Follow-up data collection is in progress. Pilot testing on intervention procedures is underway.

Project Description:

The development and socialization of emotional responsiveness of young children to the feelings and emotions of others are investigated in two research designs. In the first, the question addressed is: (1) How predictive of later affective behavior are behaviors early in life? This is a follow-up study of children observed at 1 to 2 years of age. Assessments at these ages indicated characteristic and relatively stable individual patterns of reactions to other's emotions, e.g., sympathy, avoidance, indifference, anger. What are the continuities and changes in these patterns across time? The second research design involves an intervention: Can children's emotional responsiveness be predictably altered by planned rearing interventions? Maternal rearing techniques that were found in an earlier study to be associated with empathic and altruistic child behavior (e.g., modeling of altruism and affective-inductive teaching) will be taught to mothers whose children show little positive response to emotional distress in others.

There are three samples. One sample is 22 of the 24 children studied at 1 to 2 years of age; they are now 5-6 years of age. (See project Z01 MH 00242). Second and third samples, comparable in demographic characteristics, are children (a) age-matched to the children in the initial study (i.e., 1 to 2 years) and (b) age-matched with the children at follow-up (i.e., 5 to 6 years). These samples provide replication data. They are also chosen to enable one to compare effects of intervention at two stages of development.

Observation records will be made by mothers and investigators, over a two-month period, of children's reactions to the emotional experiences of others (e.g., fear, pain, sorrow, affection). The procedures are those of previous studies(Z01 MH 00242). The data records are taped narratives, i.e., sequential accounts of emotional incidents in the child's immediate experience, the child's reactions, and the mother's behaviors relating to the incident.

On the basis of these assessments, children will be identified as low empathy and sympathy. Their mothers will be invited to be part of the intervention design to attempt to increase their children's responsiveness. Mothers will be asked to intervene by (a) modeling empathic caregiving in circumstances of distress, (b) affective-inductive teaching about the consquences of other's distress experiences, and/or (c) instructing the child to alter his/her own affective expression towards another is distress (encouraging the child to "try on" an alternative emotional expression).

Data collection has begun.

The data will be analyzed to determine (a) the stability over time of individual differences in expression of affect and in sensitivity to other's affect, (b) developmental changes in children's behavior in encounters with emotion, and (c) the effectiveness of maternal behaviors in modifying children's sensitivities to the emotional states of others.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER 02134 Z01 MH ~~00274~~-01 LDP

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)
Developmental Changes in the Understanding of Pictures Representing the Production of Sound

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Sarah L. Friedman	Research Psychologist	LDP NIMH
OTHER:	Marguerite B. Stevenson	Research Psychologist	Univ. of Wisconsin Madison, Wis.

COOPERATING UNITS (if any)
University of Wisconsin, Madison, Wisconsin

LAB/BRANCH
Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.10	.05	.05

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This investigation will examine age changes in the ability to interpret correctly pictorial representations of sound production. Sound cannot be captured in a three dimensional picture, however, the production of sound can be represented by various techniques: (1) Postures. Some postures suggest that sound is being produced; for example,a person may have his mouth open and lean toward the listener. (2) Context. Musical instruments, tools, and other objects are understood to make sound when they are held or moved in certain ways. (3) Arbitrary methods are used to suggest sound production. These methods include balloons containing words, lines or musical notes drawn next to the source of sound. Pictures using the three techniques for representing sound and matched pictures without indications of sound will be shown to subjects between 2 and 20 years of age. It is expected that there will be an interaction between age and the pictorial technique used when the correctness of interpretations is examined.

PHS-6040
(Rev. 10-76)

Project Description

A correct reading of representational pictures involves the ability to detect an equivalence between pictorial forms and their real referents. Yet, there are many differences between pictures and the reality they represent. Consider the fact that a picture is a flat surface while our world is three dimensional. The pictorial shapes very rarely match the size, color, and texture of the objects represented. Movement must be represented statically and the production of sound must be represented without sound. Despite these differences between pictures and reality, pictures are widely used as a means of communication.

In this culture children are introduced to picture books when they are as young as six months. Yet, the materials presented in picture books are not always geared to the child's picture reading abilities. For example, Friedman and Stevenson (1978) found that contemporary children's picture books make use of pictorial movement information which young children (kindergarten, first grade) ignore when classifying pictures as showing or not showing movement. In a small survey of 11 picture books with a total of 248 pictures, 39 pictures represented sound production. Currently we do not know to what extent the children for whom these pictures were designed are capable of understanding the pictures.

This investigation will examine age changes in the ability to interpret correctly pictorial representations of sound production. Sound cannot be captured in a three dimensional picture, however, the production of sound can be represented by various techniques. Some pictures suggest sound production by posture. The speaker may have his mouth open as he/she looks and gestures toward the listener. Some pictures of sound production show musical instruments, tools and other objects capable of making sound. In order to understand these pictures it is necessary to understand the ways in which these objects make sound in the environment. Other pictures of sound production use arbitrary symbols for sound. For example, in medieval art, words are drawn on ribbons coming from the mouths of angels. In contemporary cartoons, balloons with words or musical notes are drawn near mouths. We hypothesize that pictures using the above techniques vary in the degree to which they suggest that sound is being produced. Consequently we predict that the understanding of the postures suggesting sound requires the least pictorial acculturation while the understanding of arbitrary indicators of sound requires the most acculturation. Pictures using the three techniques for representing sound and matched pictures without indication of sound will be shown to subjects between 2 and 20 years of age. It is expected that there will be an interaction between age and the pictorial technique used when the correctness of interpretations is examined.

The study will provide some of the evidence that will help design appropriate pictorial materials for children of different ages. This is an important objective since in this culture pictures are important means of introducing

children to the possibility of visual representation and to reading: the decoding of visual patterns which are often quite arbitrary.

The procedures and stimuli are in the process of being developed.

PUBLICATIONS:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER 02135 Z01 MH ~~00275~~-01 LDP

PERIOD COVERED
October 1, 1977 through September 30, 1978

TITLE OF PROJECT (80 characters or less)
Children's Responses to the Emotions of Others: Naturalistic and Experimental Studies

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Marian Radke-Yarrow	Chief	LDP	NIMH
Other:	Carolyn Zahn-Waxler	Research Psychologist	LDP	NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.60	.20	.40

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The focus of inquiry is the impact on children of the emotional states of other persons. These emotions can be isolated events or chronic conditons in the life of the child. How children process these stimuli and behave in their presence and how children are affected in a cumulative way are the questions to which this research is addressed. The children are of three age groups (2 to 4, 6 to 8, 10 to 12), from different familial backgrounds. Mothers trained in procedures of reporting are the primary source of naturalistic data on children's responding to affective events in the immediate environment. Laboratory assessments of children's emotions, behaviors, and inferences in relation to structured affective events will also be made. Pilot testing is underway.

PHS-6040
(Rev. 10-76)

Project Description:

The focus of inquiry is the impact on children of emotional states of other persons. In the routine experience of children, a very large class of stimuli is emotions and feelings of others - a mother's anger, a father's depression, a sibling's pain, a peer's fear. These emotions can be isolated events or they can be "chronic" in the life of the child. How children process these stimuli and behave in their presence, and how children are affected in a cumulative way are questions that are being examined across the span of childhood. These investigations are a continuation in a program of research in which children's emotional responses to persons in distress have been studied. The previous studies have been of children in the very early years of childhood. The concern in the present research is to extend investigations to later ages and to different populations. In naturalistic procedures, mothers' observation reports will be the primary data source (See Project Z01 MH 00242-04). Additionally, investigation is extended to experimental methodology in which standard events will be used to obtain direct observational data on how children behave in the presence of emotions. Data on the kinds of inferences and reasoning about interpersonal emotional events will be obtained. Children of three age groups will be sampled: 2 to 4 years, 6 to 8 years, 10 to 12 years. Boys and girls will be equally represented. The precise family sampling characteristics are currently being worked out.

The schedule of family participation involves: (1) training of mothers, (2) four weeks of data gathering by the mother, (3) two weeks of intermission in mothers' reporting, and (4) four weeks of data gathering by mother. The experimental sessions for the child alone and for mother and child follow the in-home data collection.

Pilot testing is currently underway.

The research is directed to identifying developmental changes in the processing of emotional stimuli and to achieving a better understanding of the effects of emotional stimuli on children's functioning.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER 02136 Z01 MH ~~00276~~-01 LDP

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)
Prosocial Interaction Among Physically Handicapped and Nonhandicapped Children

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Karabelle A. L. Pizzigati	Staff Fellow	LDP NIMH
Other:	Howard A. Moss	Research Psychologist	LDP NIMH
	Ronna Finer-Berman	Guest Worker	LDP NIMH

COOPERATING UNITS (if any)
Montgomery County Public Schools

LAB/BRANCH
Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION
NIMH ADAMHA NIH Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.35	.60	.75

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X
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In the past, handicapped and nonhandicapped children traditionally have been segregated in many settings. Recent legislation, however, indicates that there will be greater integration of handicapped children, particularly in public school settings. The resulting social adjustment of handicapped and non-handicapped children to each other is a major concern. The purpose of this investigation is to study (1) the effects of physical disability on social interaction, and (2) the effectiveness of two strategies to promote positive attitudes and positive interpersonal interaction among handicapped children and their peers. Experimenters observe and code the behaviors of handicapped and nonhandicapped children ages 5-10. Observations are made in school classrooms and in small group free play situations before and after curriculum intervention. The children's behaviors are coded with respect to the amount and quality of several aspects of interaction.

PHS-6040
(Rev. 10-76)

Project Description:

The purpose of this project is twofold: First, we are investigating the differences in social interaction among physically handicapped and non-handicapped children in both segregated and integrated settings (i.e., does the incorporation of physically handicapped children in groups of nonhandicapped children substantially change how children interact with each other). Second, we are examining the effectiveness of two potential curriculum strategies to promote more positive attitudes and behaviors toward handicapped individuals. The strategies are derived from currently available school curriculum guides. Both strategies involve a curriculum approach to promote acceptance of differences through discussion and activities.

One strategy addresses the strengths and abilities of children with physical handicaps through discussion and group play activities. The aim of this procedure is to foster acceptance and respect for differences by encouraging an understanding of physical disabilities and promoting prosocial behaviors. The second strategy also aims to encourage prosocial interaction through group discussion and activities. This strategy does not specifically address any handicapping condition in discussion. It is general and may be less anxiety evoking among integrated groups of children.

The sample consists of physically handicapped and nonhandicapped children, ages 5-7 and 8-10, who are or will be in integrated settings. The children are observed in their school classrooms and in small group free play situations. The small groups consist of either integrated groups of children or nonhandicapped children. Observations are made before and after participation in one of the curriculum strategies. Control groups do not participate in either intervention procedure. All children are observed with respect to the following variables: proximity to members of the group, total amount and quality of interaction, cooperation, initiative, and affect.

This research will identify some of the problems and, more importantly, some of the social skills physically handicapped and nonhandicapped children have and use in interaction with each other. With this information, support and service personnel could work more effectively with handicapped and non-handicapped children. In addition, this study is a step in the process of developing an ongoing curriculum to promote positive attitudes and behaviors. Finally, this study addresses some of the developmental differences apparent in working with children of various ages on the same issue and will contribute to our understanding of how children learn.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 02137 ~~00277~~-01 LDP

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Constancy and Change in Personality Development

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Howard A. Moss	Research Psychologist	LDP NIMH
OTHER:	Elizabeth J. Susman	Research Psychologist	Pennsylvania State University

COOPERATING UNITS (if any)

Pennsylvania State University

LAB/BRANCH
Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION
NIMH ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.60	.40	.20

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A long standing and pervasive assumption held by lay persons and psychologists alike is that an individual's behavior is generally predictable across a variety of situations and over time. A number of longitudinal studies have been conducted over the past 50 years to test the developmental hypothesis concerning the stability of personality. Systematic patterns of change have been investigated as well as stability, although the emphasis has been on searching for patterns of stability of personality characteristics. The purpose of this project is to review and integrate the findings from the various longitudinal studies concerning the evidence for stability and change in personality over the life span. Attention is given to the assumptions associated with assessing stability and change and to the methodological and psycho-social conditions that influence whether stability or change is observed.

PHS-6040
(Rev. 10-76)

Project Description:

The emergence and course of personality characteristics over time is of considerable interest to developmental psychologists. Does a particular behavior continue to characterize the individual in a consistent manner over long periods of time? Are there predictable developmental transformations? Do certain earlier behaviors presage later, but phenotypically different, personality patterns? Are certain personality characteristics ephemeral and are others immutable; and, if so, what factors influence change? It seems patently evident that these questions can only be answered through the pursuit of long term longitudinal studies. Twelve major longitudinal studies have been reviewed with respect to the above questions.

The studies are: Oakland Growth Study, Berkeley Growth Study, University of California Guidance Study, Fels Research Institute Study of Human Development, Menninger Foundation Infancy, Coping, and Mental Health Studies, New York University Study of Behavioral Development, Terman Gifted Children, Purdue Longitudinal Study, Nesselroade and Baltes Longitudinal Study, Kelly Longitudinal Study of Married Couples, Bethesda Longitudinal Study, and Robins Longitudinal Study of Children Exhibiting Anti-social Behavior.

The personality variables examined in this review include affiliation, aggression, achievement, heterosexual behavior, sex role interests, temperament, behavioral style, dependency, passivity, psychological reactions related to stress, and maladaptive behavior. In general, little stability appears over the early months and years of life. The one exception was that vocal, smiling, and visual contact behavior of the infant toward the mother were predictive of general activity and positive interaction and affective behavior toward peers at the pre-school level for males but not females. Increased age was associated with higher and more significant stability coefficients for most of the personality variables that were studied. A number of personality variables (e.g., achievement, recognition seeking, dependency, aggression, and sex role interests) are significantly correlated with cognate variables assessed during the early adult years. There is more evidence of stability between these personality measures taken within the adult years. However, the strongest relations tend to occur between personality characteristics of contiguous ages with the magnitude of the correlations diminishing as the time intervals between assessments increase. One should expect greater evidence for stability among older subjects, since the measurements are obtained within a developmental period in which the developmental tasks and life space remain relatively constant.

Socially sanctioned behavior tended to show the greatest stability, whereas attitudes and values that are often associated with specific reference groups and ephemeral cultural positions show low stability. For example, achievement and recognition seeking behavior exhibited stability from childhood through adulthood, starting with the early school years. Dependency behaviors, which tends to be culturally sanctioned for females, was stable only for this sex from middle childhood on. On the other hand, aggression was stable just for males over the same span of time.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER 02138 Z01 MH ~~00278~~-01 LDP

PERIOD COVERED

October 1, 1977-September 30, 1978

TITLE OF PROJECT (80 characters or less)

Visual and Verbal Mode of Television Presentation and Children's Learning of Content

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Elizabeth J. Susman Visiting Fellow LDP NIMH

OTHER: None

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Developmental Psychology

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INSTITUTE AND LOCATION

NIMH ADAMHA NIH Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.10	.05	.05

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☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The study assessed the effect of a visual (camera zooms) and a verbal (verbal labels) mode of television presentation on children's learning and performance of the prosocial content. The effect of mode of presentation on visual attention to the program was also assessed. Verbal labels had a greater effect on learning and behavior and attention to the program than camera zooms. Visual attention was higher when camera zooms were absent than when they were present.

PHS-6040
(Rev. 10-76)

Project Description:

The effect of aggressive and prosocial television content on the attitudes and behavior of young children has been demonstrated in a variety of field and laboratory studies. Viewing aggression on television has been shown to increase aggressive behavior in the viewer. Similarly, prosocial content has been shown to increase sharing and cooperation among children. However, the influence of specific content is only one dimension for explaining the effect of a complex medium of television.

Mode of presentation or the combination of visual and auditory symbolic codes constituting the stimulus probably also mediates the effect of television. Auditory components include music, voice, and other sound effects. Visual techniques or visual imagery includes camera zooms, slow motion, and animation. Recent research (Salaman, 1976) demonstrated that camera zooms which highlighted important aspects of a television program enhanced learning of television content in school-age children. Identifying the effect of mode of presentation is important for television research.

Mode of presentation potentially could be used to enhance the effect of prosocial television or detract from the effect of aggressive content. The purpose of this study was (1) to determine the effect of two modes or presentation, camera zooms and verbal labeling, on learning and behavior, and of a prosocial television program; and (2) to determine the effect of mode of presentation on visual attention to the program.

The sample consisted of 40 male and 40 female preschool children. Five versions of a short television program formed the experimental conditions. In four prosocial versions preschool and school age actors shared prizes they had won on a quiz show. Camera zooms and verbal labels were varied in the programs, to form the following experimental conditions: (1) camera zooms-verbal labels. The camera zoomed in on the actors while they shared their prizes and the commentator enthusiastically labeled the sharing behavior; (2) verbal labeling alone; (3) camera zooms alone; and (4) no verbal labels-no camera zooms. Sharing was edited out of a fifth control version. Preschool children viewed one version of the television show and were tested on measures designed to assess learning (a multiple choice content test) and performance (a sharing game) of the program theme. Conceptual learning and performance, two aspects of imitation, are assumed to be governed by different factors (Bandura, 1971).

Children in the prosocial conditions showed only slightly more learning of the prosocial theme on the content test than children in the control condition. Analyses of variance revealed no significant differences among conditions on the learning measures but there was a tendency for girls in the camera zoom-verbal labeling combined condition to demonstrate more learning than girls in the other conditions, while boys in the verbal labeling condition demonstrated more learning of content than boys in the other conditions. In a multiple regression analysis with age, experimental condition and a teacher rating of children's general prosocial behavior, age was the best predictor of learning on the content test.

On the sharing measure children in the prosocial conditions shared significantly more than children in the control condition. Children in the camera zoom-verbal labeling and verbal labeling conditions shared more than children in the other conditions. Television viewing condition was the best predictor of sharing on the performance measures. Visual attention to the program was influenced by the experimental conditions. There was no significant difference between attention in the experimental and control condition. Attention was significantly lower when camera zooms were present than when they were absent. Attention was lower in the camera zoom alone than in the other groups.

The visual and verbal mode of presentation had minimal impact on learning of content based on measures that used abstract words and pictures. Preschoolers appeared to lack the ability to engage in a logical search process in order to decipher the correct test response. The fact that visual attention was lower in the camera zoom than non-camera zoom conditions indicates that camera zooms may have interfered with learning. Mode of presentation, primarily verbal labels, had a greater effect on performance of the prosocial theme. The process whereby mode of presentation affects behavior includes at least two factors. The visual and verbal modes of presentation appeared to function as covert mediators for later behavior. Camera zooms and verbal labeling also increase the salience of relevant program content. The latter factor is important because young children have difficulty discriminating relevant from irrelevant content.

It was proposed that television affects performance of motoric acts and not conceptual learning measured by abstract symbols because of the theorized developmental lag between knowing and acting (Piaget, 1976). Symbolic activity or "knowing that" comes later in the developmental sequence than concrete acts or "knowing how." The theoretical implication of these findings is that children internalize the representational codes of the television medium to be used as covert mediators for later behavior. The implication for the producers of children's television is that emphasizing the prosocial message of television through mode of presentation leads to increases in prosocial behavior.

The data were collected prior to the investigator's employment at NIMH (August 1976).

Publications:

Susman, E. J. Visual and verbal attributes of television and selective attention in preschool children. Develpm. Psychol. In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER ZO1 MH 00478-22 LN

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Neural mechanisms in vision

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. Mishkin	Research Physiologist	LN NIMH
OTHER:	L. Ungerleider	NEI Extramural Fellow	LN NIMH
	B. Turner	Professor	Howard University

COOPERATING UNITS (if any)

Howard University

LAB/BRANCH
Laboratory on Neuropsychology

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NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.6	2.2	1.4

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The striate cortex is the source of two major, corticocortical, multi-synaptic visual pathways. One of these follows the course of the superior longitudinal fasciculus, interconnecting the striate, prestriate, posterior parietal, and dorsal prefrontal areas, and appears to be important for spatial vision. The other follows the course of the inferior longitudinal fasciculus, interconnects the striate, prestriate, inferior temporal and ventral limbic areas, and is critical instead for object vision. Our research concerns both pathways, but the accompanying report focuses on the second, or ventral one. Our studies suggest that each of the extrastriate links in this ventral pathway adds a different capability to object vision, making possible, in turn, highly efficient object perception, object memory, and object-affect associations. The sequential dependency of these behavioral functions is paralleled by the sequential dependency of the successive neural structures in the chain.

PHS-6040
(Rev. 10-76)

Project Description:

The objective of the six sub-projects described here is to define the role in object vision of neural structures outside the primary visual projection system, using behavioral and anatomical analyses. (1) Monkeys were prepared with various lesions of the lateral and medial striate cortex and the brain studied with the Fink-Heimer silver impregnation method for determining cortical projections. Results thus far have revealed a topographically organized representation of the visual field within the posterior bank and depths of the preoccipital portion of the superior temporal sulcus; this is a full-field representation that was previously unknown. Further study of these brains will make it possible to delineate the complete map of the striate-prestriate projection system. A start will be made towards increasing the precision of this map by use of axonal transport methods. (2) Monkeys are being prepared with as complete ablations as possible of the striate- receptive zone defined in (1) prior to training them on visual pattern discriminations. The results will provide a rigorous test of the hypothesis that the prestriate cortex serves as the link between the striate cortex and the inferior temporal areas. Concurrently, to determine whether even small prestriate lesions might "disconnect" inferior temporal cortex from corresponding parts of the visual field, monkeys with such lesions will be tested for discrimination ability in different fields with the aid of a fixation-monitoring device newly developed for us by the Section on Technical Development. (3) Previous ablation studies had demonstrated that stimulus equivalence between the visual half-fields depends on the convergence of their projections onto single inferior temporal neurons. Follow-up studies using the fixation-monitoring device will test whether inferior temporal neurons account for stimulus equivalence across any retinal translation, and also whether, as suspected, it is the neurons in the posterior part of inferior temporal cortex that are mainly responsible for this stimulus equivalence mechanism. (4) Monkeys with various cerebral lesions were compared on tests designed to measure one-trial visual memory for objects. Only removals within the anterior part of the inferior temporal cortex (area TE) impaired this object recognition ability, suggesting that this is the locus in which the visual trace left by previous viewing is preferentially established. By contrast, on tests designed to measure one trial memory of object-reward associations, equivalent impairments were obtained after area TE lesions and after amygdalectomy. The results thus point to object recognition and object-reward association as consecutive processes that depend in part on the sequential participation of IT cortex and the amygdala. (5) In the process of investigating the role of temporal-lobe structures in memory we have chanced on a result that is particularly exciting because it may help to resolve a long-standing puzzle concerning the neuropathology underlying the syndrome of global amnesia in man. This syndrome, which is characterized by a profound inability to remember new experiences, has been attributed in the clinical literature to destruction of the hippocampus. Yet, attempts to duplicate this syndrome in animals by removal of the hippocampus have largely failed. What we have found in our studies, both for visual recognition memory and for object-reward association memory, is that <u>if the hippocampus is removed in combination with the amygdala</u> then a profound memory loss ensues, one that is even greater than

that produced by lesions of inferior temporal cortex. The discovery not only opens up a new possibility for resolving the discrepancy between clinical and animal findings, it also provides a new insight into the neural mechanisms of memory. Pursuit of this line of study with both behavioral and anatomical methods now constitutes a major part of this project. (6) The discoveries in (4) and (5) were antedated and guided by anatomical findings indicating that inferior temporal cortex projects both directly to the amygdala and indirectly to the hippocampus via the entorhinal area. To extend and refine our knowledge of these anatomical circuits, we are investigating with both axonal degeneration and transport methods the inputs to the limbic structures of the temporal lobe. Results thus far indicate that no visual area caudal to area TE projects to the amygdala, but that all sections of area TE do, and that they do so within a circumscribed part of the lateral and basolateral nuclei of the amygdala. In addition, other sensory systems (i.e., olfactory, gustatory, somatosensory, and auditory) project to other parts of the amygdaloid complex in such a manner as to suggest that each sensory system has a dominant influence in a given part. These findings will help guide further behavioral studies into the possible mnemonic functions of the limbic system in all sensory modalities.

Scientific Significance to Mental Health Research. Vision is perhaps the best understood of the sensory modalities in relation to the functioning of the nervous system. Yet a major problem remains unsolved in vision as it does in all sensory modalities: Delineation of the events which intervene between stimulus reception at the cortex and the observed response. The demonstration that damage to the inferior convexity of the temporal lobes in monkeys produces impairment in visually-mediated behavior opened up the hitherto inaccessible area of the intracerebral processes in vision, i.e., neural activity related to vision but beyond the level of the striate cortex. Unravelling these mechanisms in vision is aiding greatly in the solution of a general problem for psychology, viz., accounting for the intervening neural processes (thought and its breakdown) in normal and abnormal behavior.

Publications:

Deuel, R. and Mishkin, M. Limbic and prefrontal contributions to somesthetic learning in monkeys. Brain Research, 132: 521-535, 1977.

Gross, C. G., Bender, D. B. and Mishkin, M.: Contributions of the corpus callosum and the anterior commissure to visual activation of inferior temporal neurons. Brain Research, 131: 227-239, 1977.

Mishkin, M. Memory in monkeys severely impaired by combined but not by separate removal of amygdala and hippocampus. Nature, 273: 297-298, 1978.

Turner, B. H., Gupta, K. C. and Mishkin, M. The locus and cytoarchitecture of the projection areas of the olfactory bulb in Macaca mulatta. J. Comp. Neurol., 177, No. 3, 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00480-14 LN

PERIOD COVERED

October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Development of brain and behavior in primates

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	P. S. Goldman	Research Physiologist	LN NIMH
OTHER:	H. E. Rosvold	Chief, Laboratory of Neuropsychology	LN NIMH
	R. M. Brown	Sr. Staff Fellow	LN NIMH
	G. E. Alexander	Commissioned Officer	LBB NICHD
	T. J. Brozoski	Associate Professor	Grinnell College

COOPERATING UNITS (if any)

Grinnell College

LAB/BRANCH

Laboratory of Neuropsychology

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INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
7.8	3.4	4.4

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A major goal of this laboratory is the elucidation of neural mechanisms underlying the development of normal behavior and equally to understand the mechanisms responsible for recovery of function and plasticity following central nervous system injuries, particularly those originating in infancy. The approach is broadly multidisciplinary, involving behavioral, anatomical, electrophysiological, endocrinological, pharmacological and biochemical techniques. The experimental subject is the rhesus monkey, which because of its protracted span of postnatal development and its remarkable capacity for restitution of function following cortical lesions of early onset is unsurpassed as a model of normal and disordered human development. The focus of these studies is on the prefrontal granular cortex, a slowly maturing neocortical region of the primate brain particularly appropriate for studies on the sequence, duration, and correlation of structural and functional events in ontogeny.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

A major goal of this laboratory continues to be the elucidation of neural mechanisms underlying the development of normal behavior and equally to understand the mechanisms responsible for recovery of function or plasticity following central nervous system injuries, particularly those originating in infancy. The approach is broadly multi-disciplinary, involving behavioral, anatomical (to be described in a separate project), electrophysiological, endocrinological, pharmacological and biochemical techniques. The experimental subject is the rhesus monkey, which because of its protracted span of postnatal development and its remarkable capacity for restitution of function following cortical lesions of early onset, is an ideal subject for studies aimed at understanding neurobehavioral specificity and plasticity. Moreover the suitability of the primate as a model of normal and disordered human development is unsurpassed. The focus of these studies is on the prefrontal granular cortex. This slowly maturing neocortical region of the primate brain is particularly appropriate for studies aimed at resolving the sequence, duration, and correlation of structural and functional events in ontogeny. The emphasis of this program of research is reflected in the titles of four projects: (A) Cortical and Subcortical Unit Activity at Different Stages of Postnatal Development, (B) Role of Experience in Recovery from Early Brain Damage, (C) Behavioral Consequences of Prenatally and Postnatally Induced Brain Damage, (D) Neurochemical Correlates of Normal and Disordered Development in Primates.

A. Cortical and Subcortical Unit Activity at Various Stages of Postnatal Development

Previous research in this laboratory has provided evidence that specific regions of the primate cortex are functionally immature at birth and that different regions develop their respective functions according to widely different time-tables. The process by which neurons differentiate specific functions still remains one of the key problems of neurobiology. In the last few years we have applied a novel approach to the study of functional maturation in specific regions of the primate brain. Instead of the conventional method of ablation, we applied the reversible lesion method of cryogenic depression to the analysis of cerebral function at different stages of development. We were successful in inactivating the cerebral cortex by local hypothermia to produce consequences similar to those produced by surgical ablation, but with the important advantage that the lesion is reversible. Using this method, we demonstrated that cryogenic depression of the dorsolateral prefrontal cortex at a temperature sufficient to induce 21 - 25% decrements in the performance of a spatial delayed response task in 34 - 36 month-old monkeys produced no detectable loss of performance in 9 - 16 month-old monkeys and only a slight decrement in performance in 19 - 31 month-olds. The spatial delayed response task is among the most sensitive and selective measures of prefrontal association cortical function. We can conclude from these results that the dorsolateral prefrontal cortex in primates develops its role in mediating delayed-response function only gradually and does not become fully functional until nearly 3 years of age,

which is time of sexual maturity in this species. These results additionally indicate that the cryogenic method can be an extremely useful approach to the problem of functional development in other areas of the brain including subcortical centers.

This year we have extended our analysis of the maturation of the dorsolateral cortex with a detailed electrophysiological study of the properties of cortical neurons in relation to performance on the spatial delayed-response task. Chronic indwelling cylinders were implanted over the dorsolateral prefrontal or the posterior parietal cortex of the brain in monkeys ranging from 9 to 36 months of age. Before surgery the monkeys were trained to perform the delayed-response task in a semi-automatic testing chamber. Following surgery, unit activity was recorded from the frontal or parietal cortex while monkeys performed the task. The major findings are: (1) neurons of the dorsolateral prefrontal cortex fire in relation to parameters of the delayed-response task in adult monkeys, (2) one class of neurons fires preferentially only during the delay phase of the task indicating a possible role in short term memory, (3) a second class of neurons fire in relation to spatial location of the bait. In the delayed response task, rewards are sometimes placed into a foodwell to the right of the animal and other times in a foodwell to the monkey's left. In the prefrontal cortex of adult monkeys, units that fire preferentially only when the left or only when the right foodwell is baited, have been clearly identified, thus indicating that they may possess a mnemonic code for "left" and "right". This selectivity is independent of hand used to retrieve the reward. (4) neurons in the parietal cortex do not exhibit a similar profile of response characteristics indicating that parietal cortex plays a different if any role in the performance of delayed-response tasks, (5) units which fire in relation to the delay or exhibit "left" and "right" preferences may be found in the dorsolateral prefrontal cortex of 9 - 12 month-old monkeys, but there are many fewer of such neurons in the younger group than in the adult. These results thus provide powerful electrophysiological evidence that the neurons of the dorsolateral cortex are immature at this stage of postnatal development. This in turn can help to explain why these neurons can be removed either by surgical resection or functional depression without producing behavioral impairments on the delayed-response tasks at early stages of postnatal life. A detailed quantitative analysis of the types and percentages of neuronal classes that can be recorded in immature as compared with mature cortex is currently in progress. Also, analysis of electrophysiological data obtained from the caudate nucleus or dorsomedial nucleus of the thalamus in chronic behaving monkeys at different stages of development and in relation to cortical depression or inactivation will allow us to analyze the nature and changes in cortical regulation of subcortical activity with age.

B. The Role of Experience in Recovery from Early Brain Damage

The importance of experience for the development of behavior and for the maturation of the central nervous system in normal individuals is widely recognized. Only recently, however, it has become clear that experience

may play an equally important though as yet poorly defined role in neuropathological development. A growing number of studies has provided evidence that recovery of function following brain injury in early life does not necessarily occur spontaneously but rather depends on the opportunities which the environment provides for stimulation during the course of postoperative development. Most of this evidence has come predominantly from studies of rodents. These studies have provided evidence that experience counteracts brain trauma, but in general, the environmental effects have not been impressive in magnitude. Recent evidence obtained in our laboratory indicates that early experience may have unexpectedly profound effects on the course of recovery from brain damage in rhesus monkeys whose cerebral organization, parameters of development, and capacity for complex behavior are more similar to those of man. The major findings may be summarized as follows:

1) The return of some abilities after brain injury in early life depends heavily upon experience. Rhesus monkeys given orbital prefrontal cortical lesions as adults typically exhibit severe impairments on a spatial alternation test. However, rhesus monkeys given the same lesion as infants can perform as well as normal monkeys when they reach two years of age, but only if they have received prior training on a series of cognitive tasks during development. Without such prior test experience, monkeys given the equivalent lesions as infants totally fail to learn the delayed alternation task when they reach two years of age.

2) The impact of formal test experience in promoting recovery is directly related to the age of the subject when the brain injury is sustained. If equivalent postoperative experience is provided for monkeys operated as infants or as adults, recovery of function is exhibited only by those operated as infants. The effect is all-or-none.

3) The effectiveness of experience in promoting recovery appears to be related to the age at which it is given. Monkeys whose orbital prefrontal cortex was resected at 2 months of age tend to achieve higher recovery scores on the spatial task if the same kind and amount of experience is allowed earlier, rather than later, in the course of their postoperative development. It is not known, however, if the greater effectiveness of the earlier experience is due to its temporal proximity to the injury or whether, in absolute terms, there exist sensitive or critical periods for these effects.

4) If brain damage is extensive or includes critical areas, recovery does not occur in spite of the opportunity for extensive postoperative test experience. A striking example is given by the finding that monkeys whose dorsolateral prefrontal cortex is removed along with the orbital cortex (prefrontal lobectomy) do not exhibit any sign of learning spatial tasks at maturity, even when given the same kind and amount of experience as that which induces recovery following orbital prefrontal resections alone. Such findings indicate that the dorsolateral prefrontal area may be a critical neural substrate for mediating the environmentally facilitated recovery of

function. Anatomical and physiological studies designed to examine this possibility are now in progress.

5) The salutary effects of experience on recovery are not reducible to training effects. To determine whether the positive consequences of early experience are due to its specific content, monkeys with orbital prefrontal lesions and unoperated controls were trained on a nonspatial task as infants and examined on both a spatial and nonspatial task as juveniles. As the nonspatial task was identical to that given in early life, the opportunity for savings as a result of direct experience was optimized for this test. The performance of the experienced monkeys was compared to that of operated and unoperated monkeys who had not received early training. Performance on both classes of task was predictably impaired by orbital lesions; however, in spite of the opportunity for test-retest savings, postoperative training in infancy ameliorated the spatial but not the nonspatial deficits caused by orbital prefrontal lesions. The evidence that recovery of spatial functions can be fostered by a variety of different training histories, including one which actually reinforces nonspatial strategies, indicates that experience may act nonspecifically to offset deficits caused by circumscribed brain damage in primates. Therefore the effects of experience in these studies do not seem to be a simple matter of specific training or incidental learning related to familiarity with the testing situation.

Although further investigations are clearly needed to unravel the relevant parameters of experience that encourage restitution of function, the evidence available from our studies on infrahuman primates indicates that experiential factors do play a role in minimizing the behavioral effects of focal brain injury sustained in early life. These studies further indicate that the effects of experience can be powerful and that experience may produce its beneficial effects by providing a general level of stimulation rather than by encouraging the entrainment of specific habits. The recognition that environmental stimulation may be a potent factor governing an organism's capacity to compensate for brain injury raises certain hopes for the clinical treatment of brain injury and also provides some clues to possible neural mechanisms responsible for restoration of function in a damaged nervous system.

C. Behavioral Consequences of Prenatally and Postnatally Induced Brain Damage

This laboratory has produced several lines of evidence establishing the young brain's superior recuperative potential. First, removal of the orbital prefrontal cortex at 1, 4 or 8 weeks of life does not produce the lasting multimodal behavioral deficits that result from identical removals in adult animals. Although, as mentioned in an earlier section, full recovery following lesions requires the facilitation of experience, this salutary effect is possible only in cases of early brain injury. Second, removal of the dorsolateral prefrontal cortex can be performed in neonatal monkeys until at least 2 months of age without producing the severe spatial-mnemonic deficits that reliably result from similar or even more circumscribed injury in adulthood. Some animals with dorsolateral lesions may exhibit

some signs of arrested development as they grow older; nevertheless such cases are still more competent than animals operated as adults. The third and most dramatic line of evidence for the resilience of the immature brain comes from our recent demonstration that the dorsolateral prefrontal cortex could be removed bilaterally in a monkey fetus two months prior to birth. Neurosurgery was performed prenatally, the animal was returned to the uterus and allowed to survive until term when it was removed by Caesarean section. Studied for nearly three years of its postnatal life, the prenatally operated monkey did not exhibit any signs of sensori-motor problems at any stage of its development and performed as well as normal animals on all tests of cognitive function that were administered over the course of its postnatal life.

All of this evidence for central neural plasticity in primates has, until now, been based largely on studies of association cortex. Such areas, it could be argued, may be more dispensable because their wiring is perhaps less rigidly specified, at least in comparison to the more rigid point-to-point topography of sensory and motor cortical systems. During the last year we have extended the evidence for plasticity of the developing nervous system to brain injuries sustained by the primary visual and primary motor cortex. In one study, the motor cortex and large areas of other perirolandic regions were removed in one hemisphere during the mid-gestational period and the fetus brought to term. The prenatally operated animal was studied neurologically during early postnatal development and found to exhibit neither weakness nor spasticity of the limbs contralateral to the partial hemispherectomy. Most significantly, voluntary motor performance seemed to be largely unaffected. The animal was able to ambulate using both limbs and to support its weight equally well by the limbs of either side of the body. Motion picture records have been made of these motor achievements. A more detailed investigation was made of the integrity of perceptual function in two monkeys in whom the entire primary visual cortex was removed in the first week of life. These animals were tested on a variety of increasingly complex pattern discriminations as well as on a number of other measures including color discrimination, discrimination reversals, and tests of visual acuity. They performed surprisingly well on all visual tasks that have so far been administered; moreover, in their home cages, they exhibit normal visual exploratory behavior for their age. In essence, the evidence is unambiguous that these two animals with either total or nearly total lack of primary visual cortex are able to see fine visual detail. Either pattern vision once thought to require primary visual cortex can be subserved by other parts of the visual or extravisual systems or anomalous connections have formed in the brains of these cases that could play a role in the unusual restoration of visual capacity. These possibilities can and will be examined by appropriate morphological studies involving transneuronal transport of radioactive isotopes to trace anomalous retino-geniculo-striate connections and by 2-deoxyglucose mapping of functional pathways during restored performance.

D. Neurochemical Correlates of Normal and Disordered Development in Primates

Neurochemical studies are being conducted in order to learn more about the

maturational status of the brain at different stages of development and to more fully understand neural and behavioral plasticity in the developing primate. Measures of neurotransmitter levels and rates of synthesis give some indication of normal development of synaptic mechanisms and provide a baseline for evaluating neural response to acute and chronic brain injury. Our studies focus on the monoamines: dopamine, noradrenaline, serotonin and its metabolite, 5-hydroxyindoleacetic acid (5-HIAA). These were measured by cation exchange chromatography and fluorimetric assay. Catecholamine and indoleamine synthesis rates were obtained by measuring the accumulation of Dopa and 5-hydroxytryptophan following inhibition of aromatic amino acid decarboxylase activity by NSD 1015. The neurochemical investigations fall into four categories: 1) regional distributions in brain of mature rhesus monkeys; 2) development of monoamines; 3) assessment of functional contribution of cortical monoamines; and 4) alteration of monoamines following brain damage.

1. Regional Distribution In Adult Monkey. Endogenous monoamine concentrations and turnover rates vary markedly in different regions of neocortex as well as in various subcortical structures of young adult rhesus monkeys. Monoamine levels and synthesis rates in amygdala, hippocampus, neostriatum, thalamus and brain stem are generally similar to comparable measures previously reported in a variety of species. However, cortical monoamines exhibit topographically specific patterns of distribution that have not been described before. Thus, dopamine concentration is highest in the prefrontal and temporal neocortex; it decreases along the fronto-occipital axis and only trace amounts are detectable in the visual cortex. The distribution of norepinephrine is similar to that of dopamine except that the highest concentrations of norepinephrine are found in somatosensory cortex instead of prefrontal cortex. The pattern of distribution of serotonin is more uniform. However, the distribution of its metabolite, 5-hydroxyindoleacetic acid, is complementary to that of dopamine: the concentration is lowest in prefrontal cortex and highest in posterior regions of the telencephalon.

Synthesis of catecholamines as measured by DOPA accumulation in monkeys treated with an aromatic amino acid decarboxylase inhibitor, NSD 1015, generally parallels the distribution of the catecholamines while indoleamine synthesis as measured by 5-HTP accumulation is similar to the distribution of 5-hydroxyindoleacetic acid. It may be significant that synthesis rates for the catecholamines are especially high in various areas of association cortex. Until now there has been little recognition of the differential concentrations and turnover of monoamines in various areas of primate cortex. These findings as well as the evidence for differential rates of development (see below) encourage the detailed examination of the neurochemical basis of higher behavioral processes and their elaboration. In particular, the role of serotonin in visual function, norepinephrine in somatic sensation, and dopamine in cognition need to be explored.

2. Development of Monoamines. Studies on the development of monoamine levels and rates of synthesis in major cortical and subcortical subdivisions of the primate nervous system reveal that there are considerable differences in the time-tables of development for dopamine, norepinephrine and serotonin

in different regions of the nervous system. We have identified three major periods of maturation which span a) a neonatal stage from birth through 8 months of age; b) a juvenile stage from 8 months to 2-3 years of age; and c) maturity which begins at 2-3 years and extends to 15 years and over, which in rhesus monkey can be considered the equivalent of old age in man. Since the changes in brain chemistry with age are quite complex, it will be convenient to describe the major findings for each putative transmitter separately. Dopamine is found in high concentrations in the prefrontal cortex of newborn monkeys. However, the level and rates of synthesis of this amine decline over the neonatal period from birth through 8 months of age. In contrast, dopamine content and activity in motor cortex increase over the same period, reaching a peak by 8 months of age. The decline in prefrontal dopamine and the increase in precentral dopamine may possibly reflect a redistribution of dopaminergic activity within the frontal lobe over the neonatal period of postnatal development as well as maturation of control mechanisms for dopamine synthesis and regulation. Peak-levels of dopamine that are attained in motor cortex in the first eight months may be related to the increase in motor dexterity and fine coordination that begins to be evident at this age. Dopamine content and activity in prefrontal cortex increases from 8 months to 2-3 years of age followed by a marked decrease in aged monkeys. The increase in prefrontal dopamine at the stage of sexual maturation correlates with our independent evidence from studies of surgical and reversible lesions and from electrophysiological data which indicate that the dorsolateral prefrontal cortex reaches maturity for certain cognitive functions at this age. The decline in dopamine in old-aged animals (which is restricted only to prefrontal cortical dopamine) may be related to the decline in cognitive efficiency that sometimes occurs in old age. If so, reversal of such disorders may be possible with dopamine agonists.

In contrast to dopamine, norepinephrine exhibits a steady and graded increase with age in both levels and biosynthetic activity in all areas of the brain where it is present in measurable concentrations. It is noteworthy that norepinephrine which is present in peak concentrations in somatosensory cortex at all ages falls off sharply in the 15 year old monkeys. Norepinephrine content of other cortical and subcortical regions is only minimally affected in old age.

Serotonin is rather uniformly distributed throughout the neocortex at all ages but its metabolite, 5-HIAA, is present in higher concentrations in posterior regions of the neocortex. Developmentally, serotonin synthesis rates are low in parietal and visual cortex over the neonatal period from birth through 8 months. However, between 8 months and 2-3 years, there is a 75% increase in synthetic activity in these regions, thus indicating an increasing sertonergic influence of the functions of parietal and visual cortex with maturity.

While the functional role of monoamines in neocortical activity and in development of such activity needs to be elucidated, the dynamic and regionally specific changes in monoamine concentration and activity suggests a differential role for the different transmitters in each area of the

cortex. Further, the functions mediated by each cortical area may well be modulated by a different neurotransmitter at each developmental time period. Further studies are required to decipher the meaning of these shifts in neurotransmitter balance over various stages of the life span and to explore their significance in pathological as well as normal behavioral development.

3. Functional Contribution of Cortical Monoamines. Selective depletion of neurotransmitters in a specific region of the brain has been accomplished by intracerebral injections of specific neurotoxins into a circumscribed region of the cerebral cortex in monkeys trained to perform tasks dependent on the functional integrity of the area in question. For these studies, we have focused on the dorsolateral prefrontal cortex because of its selective role in the mediation of spatial delayed-response problems which can be reliably measured and also because this area of cortex is characterized by notably high levels and rates of synthesis of catecholamines, particularly dopamine. Following intracerebral microinjections which were intended to cause relative depletion of noradrenaline (6-OHDA), dopamine (6-OHDA plus systemic desmethylimipramine) serotonin (5,6-dihydroxytryptamine) as well as injections of saline, monkeys were tested on delayed alternation visual discrimination problems in a manner identical to preoperative training. They were additionally tested under the influence of various drugs selected for their properties as catecholamine agonists. At the completion of postoperative testing, each animal was sacrificed and its brain dissected for subsequent cation exchange chromatography and fluorimetric assay of serotonin, 5-HIAA, dopamine and noradrenaline. The major finding so far obtained is that depletion of dopamine but not that of norepinephrine or serotonin causes severe impairment in delayed alternation performance. Of particular significance, the deficit can be reversed pharmacologically with the dopamine agonists, L-DOPA and apomorphine. These findings provide a) the first evidence of a specific role of a neurotransmitter in a specific cortical function and b) a promising basis for the study of the therapeutic possibilities of drugs in recovery from cortical brain damage or biochemical deficiencies.

4. Alteration of Monoamines Following Brain Damage. To investigate the hypothesis that the developing brain reacts differently to damage than a fully mature brain, levels and synthesis rates of monoamines in various cortical structures have been measured eight months following bilateral orbital lesions in infant and adult monkeys. The monoamine levels and synthesis rates have been compared to data from normal animals of the same age group. Two major differences in response to damage have so far been observed between infant-lesioned and adult-lesioned animals: a) At the site of damage, there is an increase in catecholamine synthesis in infant brains, and a decrease in synthesis in adult brains. b) In thalamus of the infant-lesioned brain, there is an increase in dopamine content and a 50% decrease in serotonin synthesis rate. A different response occurs in the thalamus of adult-lesioned brain. Here dopamine content does not increase and the serotonin synthesis rate increases 200%. Further work is in progress to identify the biochemical alterations that occur following brain damage. The findings from such studies may eventually suggest a pharmacological approach to ameliorating some of the symptoms following brain

injury in the very young and the old.

PUBLICATIONS:

Goldman, P. S. and Mendelson, M. J.: Salutary effects of early experience on deficits caused by lesions of frontal association cortex in developing rhesus monkeys. Exp. Neurol. 57: 588-602, 1977.

Goldman, P. S. and Lewis, M. E.: Developmental biology of brain damage and experience. In Cotman, C. W. (Ed.): Neuronal Plasticity. Raven Press, New York, 1978, 291-310.

Alexander, G. E. and Goldman, P. S.: Functional development of the dorsolateral prefrontal cortex: an analysis utilizing reversible cryogenic depression. Brain Res. 143: 233-249, 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00481-07 LN

PERIOD COVERED

October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Pre- and post-natal genesis and plasticity of connections in the primate brain.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	P. S. Goldman	Research Physiologist	LN NIMH
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COOPERATING UNITS (if any)

Howard University

LAB/BRANCH

Laboratory on Neuropsychology

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.8	0.8	4.0

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The primary purpose of this research is to describe the normal course of prenatal genesis and postnatal differentiation of connections in the brain of an infrahuman primate and to search for evidence of neuroplasticity, i.e., anomalous anatomical pathways resulting from early brain damage that would be capable of mediating behavioral recovery in primates using a variety of techniques including intrauterine surgery, horseradish histochemistry and autoradiography for tracing central nervous system connections.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

The twofold purpose of this research is to study the prenatal genesis and postnatal differentiation of normal connections in the brain of an infrahuman primate, and to search for evidence of anomalous pathways resulting from early brain injury that may be involved in sparing or recovery of function in primates.

To accomplish these goals, which are interrelated, rhesus monkeys of different fetal and postnatal ages are subjected to various neurosurgical procedures. For intrauterine surgery, a pregnant rhesus monkey carrying a fetus of known gestational age is subjected to a laparotomy and hysterotomy, the fetus is exposed through the uterine incision, neurosurgery is performed on the fetus and it is returned to the uterus and allowed to survive for several days, weeks or months and often until term as required by particular investigations. Surgical procedures performed on both fetuses and postnatal animals include injections of radioactive isotopes for tracing efferent projections, horseradish peroxidase (HRP) for both efferent and afferent axonal transport and finally, surgical resections of brain tissue have been performed on fetuses with subsequent recovery of healthy specimens at term by Caesarean section. After varying time intervals the brains are subsequently processed by autoradiographic and histochemical procedures. Often multiple procedures are performed either simultaneously (double labeling of HRP and labeled amino acids) or successively (surgical resection prenatally, isotope injection postnatally).

Two major projects have been conducted this year on the following topics: A) Prenatal Genesis of Frontal-Lobe Connections and B) Rearrangement of Connections following Brain Injury.

A. Prenatal Genesis of Frontal-Lobe Connections.

In previous studies we discovered that the associational and commissural efferents of the frontal cortex in newborn monkeys terminate in distinct columnar patterns similar in certain respects to thalamo-cortical projections in sensory systems. The projections of the frontal cortex upon the neostriatum were also found to be stereometrically organized, particularly in the head of this nucleus. Studies of other investigators on the genesis of connections in the visual system have revealed that initially thalamo-cortical fibers are not segregated into columns as they are in the mature brain but instead are intermixed, both at the level of the lateral geniculate body as well as in layer IV of the primary visual cortex. Segregation into separate territories begins only during the second half of gestation, continues in the immediate postnatal period and is not completed until about three weeks of age. It is not known whether columniation in the projections of the frontal lobe develop in the same way. Since such connections belong to a different class (cortico-cortical, cortico-subcortical rather than thalamo-cortical) and arise from an entirely different part of the brain it is of some interest to determine whether the development of this class of nonsensory connections obey the same principles and rules of development as sensory neurons. Thus, the following questions have been addressed:

1) What is the time-table of and sequence of development of associational, callosal, and cortico-subcortical (cortico-striatal, cortico-thalamic, cortico-pontine, cortico-bulbar, cortico-spinal) connections in the central nervous system of primates?

2) Do diffuse terminations precede columnar distribution of efferents in cortical and subcortical targets of frontal association efferents as they do in the development of connections in the visual system? If so, what is the time-table of appearance of such columns in the various target areas?

3) When do thalamo-cortical, and meso-cortical connections of the frontal lobe develop and what is the interaction if any between the maturation of descending and ascending projections?

To answer these assorted questions, fetal monkeys ranging in age from 84 to 124 days of gestation (which in rhesus monkey lasts 165 days) have been injected with tritiated amino acids, horseradish peroxidase or a combination of the two into the prospective dorsolateral prefrontal cortex at selected gestational ages. The fetuses were replaced in utero and following a period ranging from 1 - 3 days, they were delivered by Caesarean section and perfused through the heart with appropriate fixatives for subsequent processing for autoradiography or horseradish peroxidase histochemistry or both.

Results are not yet available from these studies due to the long term required for processing of brain tissue for autoradiography. However, preliminary findings indicate that the studies have succeeded technically.

B. Rearrangement of Connections Following Brain Injury

Rearrangement of synaptic connections is potentially the most important biological mechanism underlying recovery of function following brain injury. Most of the evidence for such rearrangement in mammals has been obtained from studies involving focal ablation of sensory, motor and limbic structures in both developing and mature rodents and carnivores. It is not known, however, whether and to what degree such neuronal plasticity can occur in the primate brain at maturity or at any stage of development. Primates, including man, exhibit remarkable sparing of behavioral function following circumscribed brain injuries, particularly those occurring early in life. Knowledge of the capacity for axonal redistribution in this species is essential for understanding the mechanisms of both reversible and permanent consequences of brain damage in man.

To analyze the possibilities for neuronal rearrangements in a primate, unilateral resection of the frontal lobe in the region of the prospective dorsolateral prefrontal cortex was performed on a fetal rhesus monkey 6 weeks prior to birth on the 119th embryonic day. The fetus was exteriorized following laparotomy and hysterotomy. Cortical tissue in the inferior bank of the primordial arcuate sulcus and in both banks of the developing principal sulcus as well as all cortex lying on the dorsal convexity superior to the principal sulcus was ablated. After the resection was completed, the fetus

was returned to the uterus and subsequently delivered around term which on the average occurs at E165. On the 5th postnatal day, a single injection of a mixture of ^{3}H-proline and ^{3}H-leucine (35μC/μl) was placed in the middle of the dorsal rim of the principal sulcus in the hemisphere opposite to that resected prenatally. One week later the monkey was sacrificed, its brain embedded in paraffin, sectioned at 10 μ and processed for autoradiographic analysis of axonally transported radioactive label. Two additional monkeys that had not undergone prior surgical procedures were injected with similar quantities of tritiated amino acids into the same region of cortex at 5 days and sacrificed one week later. Autoradiograms from these animals provided data on normal prefrontal connections in neonatal monkeys.

When the dorsolateral prefrontal cortex in one hemisphere of a rhesus monkey is resected 6 weeks before birth and the fetus survives to postnatal ages, neurons of the corresponding cortex in the unoperated hemisphere issue a greatly expanded projection to the contralateral caudate nucleus in addition to a normal projection to the ipsilateral caudate. The enhancement of the crossed prefronto-caudate pathway following prenatal neurosurgery provides the first direct evidence for lesion-induced neuronal rearrangement in the primate telencephalon.

The dramatic structural rearrangement described is relevant to the evidence for sparing or recovery of function following brain damage incurred during early stages of development, particularly for lesions sustained unilaterally. The finding in a primate that neurons from an intact neocortex of one hemisphere can expand their terminal distribution to structures of a damaged hemisphere may provide a long awaited clue to a possible neural mechanism for sparing of associative and linguistic competence following unilateral brain damage or hemispherectomy in man.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER ZO1 MH 02031-02 LN

PERIOD COVERED

October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Neural mechanisms in spatial perception

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.4	0.6

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The striate and prestriate cortex project not only ventrally, to the inferior temporal area, but also dorsally, to the posterior parietal and dorsal prefrontal areas. This dorsal projection pathway appears to constitute the visual contribution to a system for spatial perception. Many modalities undoubtedly contribute to this spatial system, which can be divided into a posterior (parietal) portion serving allocentric localization, and an anterior (prefrontal) portion serving autocentric localization. The entire system is probably critical for the spatial guidance of movement, and in keeping with this its major cortical outflow appears to be to the motor system.

PHS-6040
(Rev. 10-76)

Project Description:

The first two sub-projects described below deal with the parietal and prefrontal portions of the spatial system, respectively.

(1) Animals trained on a visual landmark test, a measure of allocentric localization ability, were prepared with posterior parietal removals in one hemisphere and complete striate removals in the other, and these were then followed by callosal transection. Results indicate that the crossed lesions alone produced severe impairment and that, once the animal recovers, the addition of a callosal transection had only a mild effect. Thus, the posterior parietal cortex, like the inferior temporal, appears to depend heavily on striate input for its visual function; but unlike the inferior temporal, the posterior parietal does not seem to receive a heavy visual input via the corpus callosum. In a second experiment, monkeys received bilateral lesions of the striate areas representing either central vision (lateral striate) or peripheral vision (medial striate). They were tested both on a pattern discrimination task, to assess residual inferior temporal function, and on the landmark task, to assess residual posterior parietal function. On the former task, only the lateral striate lesion produced a deficit, whereas on the latter task, the two lesions produced equally severe deficits. Apparently, inputs from central vision are especially important for the pattern recognition functions of inferior temporal cortex, but inputs from central and peripheral vision are equally important for the visuospatial functions of posterior parietal cortex. Thus, although interactions with striate cortex are critical for the parietal just as for the temporal area, the striate inputs to these regions appear to be organized differently, presumably reflecting differences in the cortical processing required for spatial vs object vision.

(2) A crossed-lesion experiment like that described above has shown that the dorsal prefrontal cortex also depends on striate input for its visual function, in this case autocentric localization as measured by the spatial delayed response test. Evidence that the function is selectively spatial comes from an experiment in which animals with selective prefrontal removals were compared on a series of nonspatial visual memory tests, including delayed object alternation, delayed color matching, and delayed object matching. In every case, animals with lesions confined to the principal sulcus relearned quickly, in sharp contrast to their permanent failure to relearn spatial memory tasks. The dimensions of the spatial deficit were examined in another experiment which compared the effects of selective prefrontal lesions on up-down delayed alternation as opposed to the customary left-right delayed alternation. In every case, lesions known to impair performance on left-right tasks (e.g., principalis removals, or inferior frontal ablations) had just as severe an effect on the performance of the up-down task, while removals known to have little effect on left-right alternation (e.g., periarcuate lesions) also had little effect on the up-down version. Apparently, the spatial impairment after principalis lesions is not limited to left-right confusion, but encompasses the vertical dimension as well.

(3) In another project (Neural mechanisms in vision) evidence was reviewed showing that visual discrimination habits depend on both the cortical visual system and ventral limbic structures. But are these two systems acting together a sufficient substrate for such habits, or does the rest of the cortex, including that in the dorsal "spatial" system, also play a significant role? To examine this question we restricted vision to a single hemisphere (by visually deafferenting and disconnecting the other hemisphere) and then removed all the cortex from the seeing hemisphere except for the visual and limbic systems. Unexpectedly, the operated animals appeared on awakening to be completely blind, and this total blindness lasted for one to several months, abating, if at all, only very slowly and only in part. Yet, the geniculostriate pathway in these animals seemed to be intact as judged both by visual evoked potential studies and from the subsequent histological examination of the brains.

To determine whether the blindness was the result of a disconnection of the visual system in one hemisphere from the motor system in the other, animals were prepared as before but with the forebrain commissures left intact. Yet these animals too were blind, indicating that the blindness is not a disconnection effect and suggesting further that during blindness the visual signal is not processed beyond striate cortex, for otherwise the signal should have been transmitted across the preserved commissural channels (which start at the striate-prestriate border) to the motor system. The analysis implies that the territory ablated normally provides some activating input, direct or indirect, that is necessary for cortical visual processing.

In a further study, the original lesion was subdivided in an effort to localize the area supplying the activating input. It was found that a sensorimotor lesion (Broadmann's areas 1-6) failed to produce blindness in any of three monkeys. The complementary lesion, however, consisting of dorsal prefrontal, inferor parietal,, insula and superior temporal areas, did produce blindness in three monkeys for about a month. These localization results provide additional evidence that the blindness is not a visual-motor disconnection effect, and suggest instead that it is a profound form of visual neglect, since parts of the lesion that produced blindness are known to produce neglect. Accordingly, the basis of the neglect too could be impaired processing within the cortical visual system due to loss of activating input, though a less complete loss than that producing neglect. It appears from these results that the dorsal "spatial" system normally provides an activating input (relayed perhaps through the activating system) that is necessary for the visual system and perhaps other sensory systems to work properly, and that this is in addition to its contribution to spatial mechanisms. For when the animals eventually do recover their use of vision, they then show permanent impairment in the ability to guide their body and limbs to visual targets; that is, they then show the expected spatial disorientation.

Significance to mental health. Spatial orientation in animals is an extremely well developed, higher order, perceptual mode. It is "higher order" in the sense that it depends not on a single sensory modality but on the coordinated

functioning of several of them. Our evidence points to a dual cortical localization for this suprasensory modality in the posterior parietal and dorsal prefrontal areas. Analysis of this complex, multimodal, cognitive system in monkeys may help us to understand the organization of yet another complex, multimodal, cognitive system in man, namely, the one for language. The formal similarities between the language system (for which man's left hemisphere is specialized) and the spatial system (for which his right hemisphere is specialized) give added support to the validity of this approach.

Publications:

Mishkin, M. and Manning, F. J.: Nonspatial memory after selective prefrontal lesions in monkeys. Brain Research 143: 313-323, 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER ZO1 MH 02032-02 LN

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Neural Coding of Visual Stimuli

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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OTHER:	M. Mishkin	Research Physiologist	LN NIMH
	J. Levinson	Professor	University of Maryland

COOPERATING UNITS (if any)

University of Maryland

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.4	0.6	0.8

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The results of our previous electrophysiological studies have shown that cells in the inferior temporal cortex seem more concerned with the physical properties of stimuli than with their motivational significance. In those studies we were unable to identify the stimulus parameters that seemed critical in determining the responses of the cells. Thus, a central question that remained unanswered is that of stimulus coding - to what characteristics of the visual world are cells in the IT cortex particularly sensitive. Recent studies have suggested that the visual system may be particularly sensitive to the spatial frequency components of and contrast within objects - i.e. the distances between and differences in brightness of light and dark areas. Our proposed study of the contrast sensitivity function of normal monkeys and those with selective lesions in the visual pathways is now almost fully instrumented. It is our hope that the results of this lesion study will suggest future directions for our electrophysiological investigations.

PHS-6040
(Rev. 10-76)

Project Description:

Our recent electrophysiological investigations of cells in the inferior temporal cortex have raised a number of questions whose answers are critical to an understanding of the processes by which visual objects are analyzed. One of these questions is that of stimulus coding; i.e. to what parameters of the visual world are cells in the inferior temporal cortex particularly sensitive? An answer to this question requires the kind of stimulus control that can be provided only by computer-generated displays which would allow manipulation of the stimulus in an effort to find the "best" one for the cell. Consequently, these critical studies must be postponed until the laboratory can be equipped with a computer and display device. We have therefore decided to approach the question of encoding in another way.

Over the past decade psychophysical data have accumulated which appear to support a theory of pattern perception based on the application of Fourier's theorem to the visual system. These data suggest that pattern vision may rest on the ability of the visual system to analyze objects in terms of their component spatial frequencies - i.e. the visual system appears to be sensitive to the spacing between light and dark areas and to differences in contrast (brightness) between these areas. Further, a theory of spatial frequency analysis has suggested that the visual system has multiple spatial frequency channels, each sensitive to only a portion of the total spectrum of frequencies which we can see. Thus, within certain limits each channel independently processes a different spatial frequency component or a range of spatial frequency components of a complex pattern.

The proposed investigation deals with the role of the inferior temporal and lateral striate cortex in processing such spatially patterned information. In the rhesus monkey, studies from this laboratory have shown that bilateral ablation of the inferior temporal cortex, particularly the posterior portion, produces severe deficits in pattern discrimination learning. By contrast, bilateral ablation of lateral striate cortex produces relatively mild deficits on similar tasks. However, only the lateral striate lesions produce central field defects and a consequent loss in acuity. A satisfactory explanation of this paradox in terms of spatial frequency analysis may be possible.

In general the high frequency components of a pattern are responsible for the sharp edges that produce the fine detail of that pattern. Since the integrity of lateral striate cortex is necessary for the resolution of fine detail (normal visual acuity) it appears that this part of the visual system may be particularly sensitive to high spatial frequencies. On the other hand, a model of the visual system suggested by Kabrisky suggests that cortical areas beyond striate cortex may function as low-pass spatial filters responsible for processing the overall configuration of an object. Thus, perhaps the absence of low spatial frequency analysis is responsible for the severe deficits in pattern discrimination learning following lesions of the inferior temporal cortex. Moreover, just as the small receptive fields of

lateral striate cortex appear well suited for the processing of high spatial frequencies and, therefore, for the resolution of fine detail, the relatively large receptive fields of cells located in inferior temporal cortex would appear to be well suited for the processing of low spatial frequencies, and therefore perhaps for analyzing the configurational information of overall contour.

In order to test the foregoing hypotheses, we have now almost fully instrumented, with the help of the Section on Technical Development, a study of contrast sensitivity functions to be carried out with normal monkeys and those with lesions of the lateral striate or inferior temporal cortex. The study requires generation of sine-wave gratings displayed on an oscilloscope screen; a microprocessor unit to allow automation of the entire study, including stimulus presentation and sequencing, delivery of reward, and data acquisition; and a specially-constructed animal chamber which permits optimal viewing conditions.

If these lesion studies reveal differential sensitivities to spatial frequencies, we will use that information to study single unit response not only to different gratings, but to patterns from which high or low spatial frequency components have been selectively filtered.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 02033-01 LN

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Functional Mapping of Sensory System in the Primate

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	C. D. Jarvis	Research Psychologist	LN NIMH
OTHER:	M. Mishkin	Research Physiologist	LN NIMH
	C. Kennedy	Guest Worker	LCM, NIMH
	M. Shinohara	Visiting Associate	LCM, NIMH
	M. Miyaoka	Visiting Associate	LCM, NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory on Neuropsychology

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.4	1.8	0.6

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Converging evidence from studies of lesion effects, anatomy, and single units indicates that, in the primate, the system for processing information about visual objects (i.e. object vision) extends beyond the striate cortex to include the circumstriate and inferior temporal areas. From the inferior temporal areas, in turn, information about these objects appears to be transmitted to subcortical structures in the temporal lobe. We have attempted to map this functional system by means of the 2-deoxyglucose technique, an autoradiographic method for measuring local cerebral glucose utilization (LCGU). To achieve this, we compared LCGU values in a surgically "blinded" hemisphere and in the opposite "seeing" hemisphere in the same monkey while the animal was visually active. Cortically, we found reduced glucose utilization in the blind as compared to the seeing hemisphere not only in the geniculostriate system, but throughout the entire expanse of circumstriate and inferior temporal cortex as far forward as the temporal pole. The functionally depressed areas also included tissue thought to be important in spatial, rather than object, vision.

PHS-6040
(Rev. 10-76)

Subcortically, side-to-side differences were seen in structures associated with processing information about both visual objects and visual space. The results not only provide striking confirmation of an extensive cortical visual system but also help to reveal its full extent and to localize its points of contact with subcortical structures.

Project Description

Neural structures involved in the processing of visual information have been painstakingly identified through the use of very time-consuming lesions, electrophysiological and anatomical techniques, and yet, the full extent of the visual pathway has probably not been revealed. These techniques have been used to describe a sequential system for processing information about visual objects which begins in the geniculostriate pathway and continues through the prestriate cortex along a cortico-cortical route to the inferior temporal cortex. Information is then transmitted to limbic and striatal structures, although the precise termination of this system in those areas is unclear. These techniques have also been used to identify another system, specialized to process information about spatial vision, which also depends on a geniculostriate input and a cortico-cortical pathway, in this case through the prestriate, parietal and frontal cortex. In addition, both systems are known to have subcortical inputs to, and projections from, structures in the tectofugal pathway.

We have attempted to map these functional systems by means of the 2-deoxy-glucose technique, an autoradiographic method for measuring cerebral glucose utilization (Kennedy et al., Science, 187:850, 1975). To achieve this, we prepared monkeys with a unilateral optic tract section combined with section of the forebrain commissures, thus visually deafferenting one hemisphere while leaving the other intact. This made it possible to compare values for local cerebral glucose utilization in a "seeing" and "blind" hemisphere within the same animal, and thereby map the visually related areas.

We also prepared monkeys with an optic tract section alone. In these monkeys, any information coming into the "blind" hemisphere would be received by way of the corpus callosum and anterior commissure. Optical densities would then reveal the pattern of these interhemispheric connections.

Monkeys in this initial study sat in a primate chair while visual patterns mounted on a drum rotated around their heads. They were injected with a pulse of radioactively-labeled 2-deoxyglucose and monitored over the next 45 minutes to determine the levels of free deoxyglucose in the blood. Most of the radioactively-labeled substance is incorporated into active cells during this period and any extracellular deoxyglucose is of low enough concentration so that its presence does not contaminate the autoradiographs.

In a second study, monkeys with both an optic tract section and split of the forebrain commissures were trained on a visual discrimination task using the hand opposite the blind hemisphere. The task was a pattern discrimination in which a positive stimulus was paired with one of a series of negative stimuli

in sequential blocks of trials. Correct responses to the positive were reinforced with a liquid reward. No reward followed correct responses to the negative simulus.

In both behavioral situations, reduced glucose utilization in the blind as compared with the seeing hemisphere was seen cortically not only in the geniculostriate system, but throughout the entire expanse of circumstriate and inferior temporal cortex as far forward as the temporal pole. The functionally depressed zone included tissue adjacent to the inferior temporal cortex in the upper bank of the superior temporal sulcus and in the fusiform and perirhinal areas. Subcortically in the temporal lobe side-to-side differences were seen in lateral and dorsal amygdala, ventral putamen, ventral claustrum, and tail of caudate. Outside this stimulus-processing system and its subcortical projections, asymmetries were also seen in the superior colliculus, inferior and lateral divisions of the pulvinar, and lower bank of the intraparietal sulcus and exposed surface of the inferior parietal lobule. Performance on the discrimination task led to a symmetrical increase of glucose utilization in structures associated with the active hand and to a symmetrical increase in structures associated with the act of drinking; within the visually related areas, however, the effects of the two different-testing situations were substantially the same.

The results of these studies in the visual system have provided in a short period of time such striking confirmation of the extent and the locus of visually related areas that we now plan to undertake similar experiments involving other sensory systems for which (unlike vision) the central processing pathways are still virtually unknown.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00471-23 LPP

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Studies of Heredity and Environment in Schizophrenia

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	D. Rosenthal	Chief, Section on Genetics of Mental Illness	LPP NIMH
OTHER:	P. H. Wender	Professor of Psychiatry	University of Utah
	S. S. Kety	Professor of Psychiatry	Harvard Medical School
	S. Nagler	Research Psychologist	Institute for Research on Kibbutz Education (Israel)
	F. Schulsinger	Co-Director	Psychological Institute (Denmark)
	O. W. Quinn	Guest Worker	LPP NIMH

COOPERATING UNITS (if any)
Harvard Medical School, Institute for Research on Kibbutz Education, and Psychological Institute

LAB/BRANCH
Laboratory of Psychology and Psychopathology

SECTION
Section on Genetics of Mental Illness

INSTITUTE AND LOCATION
NIMH, ADAMHA, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
6.0	3.1	2.9

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The project is composed of the following studies: (1) A constructively critical, hypothesis-oriented analysis of the worthwhile literature on heredity and environment in schizophrenia. (2) An intensive multidisciplinary study of a family with MZ quadruplets (daughters) concordant as to schizophrenia but discordant as to severity and outcome. This study was published in book form. We are continuing our contacts with this family to see what happens in the clinical course of these girls and how the course is related to earlier and current life experiences. (3) Studies of adoptees and their biological and adoptive families. (4) A study of children (of schizophrenic and control parents) reared in town or kibbutz in Israel.

PHS-6040
(Rev. 10-76)

Project Description:

The project is composed of the following studies: (1) A constructively critical, hypothesis-oriented analysis of the worthwhile literature on heredity and environment in schizophrenia. (2) An intensive multidisciplinary study of a family with MZ quadruplets (daughters) concordant as to schizophrenia but discordant as to severity and outcome. This study was published in book form. We are continuing our contacts with this family to see what happens in the clinical course of these girls and how the course is related to earlier and current life experiences. (3) Studies of adoptees and their biological and adoptive families. (4) A study of children (of schizophrenic and control parents) reared in town or kibbutz in Israel.

The objectives of this project are to understand how hereditary and environmental factors interact to make for schizophrenic outcomes of varying types and degrees.

The analysis of the literature has been reported in a series of papers and in the study of the quadruplets. A textbook which organizes and explicates the literature on the genetics of behavioral disorders and a modified version of this book have been published. Several papers on our adoption studies are now published. The studies indicate that hereditary factors contribute to the development of schizophrenia and to other disorders that we include in a group called the schizophrenia spectrum. Several articles are in preparation, and eventually a monograph will be written on the adoption studies. Data analysis is continuing. One hundred subjects in the Israel study have been examined, and the research findings are currently being analyzed and evaluated. Preliminary findings indicate that offspring of schizophrenics have more neuropathological signs in childhood than do controls. Four subjects have had a breakdown, and all have a schizophrenic parent.

It is significant to bio-medical research and the program of the Institute that we have resolved a chronic, critical problem in psychiatry by demonstrating beyond any reasonable doubt that genetic factors are importantly involved in the etiology of schizophrenia and related mental disorders. Moreover, the evidence thus far suggests that the mode of genetic transmission is polygenic or a dominant single gene with polygenic modifiers.

The proposed course of our project is to examine additional subjects in Denmark with the following goals in mind: (1) to try to discriminate gene carriers and non-gene carriers with respect to personality and test variables; (2) to compare the fate of gene carriers who are reared in the parental home and compared to those reared in adoptive homes; (3) to compare Ss who have a psychotic biological parent but who are reared by nonpsychotics with Ss whose biological parents are not psychotic but who are reared by a psychotic adoptive parent; (4) to find the incidence of schizophrenia spectrum disorder in a random sample of the Danish population. In Israel, our goal is to prepare a volume on our findings and their theoretical implications. During this year we are carrying out reexaminations of this unique sample of subjects.

Publications:

Fuccillo, D.A., Kurent, J.E., Manne, S.H., Rosenthal, D., Beadle, E., and Sever, J.L.: Lack of association between defective delinquents and antibody of herpesvirus hominis. Neurology 27: 304-305, 1977.

Kety, S.S., Rosenthal, D., and Wender, P.H.: Genetic Relationships Within the Schizophrenia Spectrum: Evidence from Adoption Studies. In Spitzer, R.L. and Klein, D. (Eds.): Critical Issues in Psychiatric Diagnosis. New York, Raven Press, 1978, pp. 213-223.

Rosenthal, D.: The Schizophrenia Spectrum Disorders: Implications for Psychiatric Diagnosis. In Akiskal, H.S. and Webb, W.L. (Eds.): Psychiatric Diagnosis. New York, Spectrum Publications, Inc., 1978, pp. 167-177.

Wender, P.H., Rosenthal, D., Rainer, J.D., Greenhill, L., and Sarlin, M.B.: Schizophrenics' adopting parents. Arch. Gen. Psychiat. 34: 777-784, 1977.

Rosenthal,D.: Genetic Aspects of Schizophrenia. In van Praag, H.M., Lader, M.H., Rafaelsen, O.J., and Sachar, E.J. (Eds.): Handbook of Biological Psychiatry, Volume II, Brain Mechanisms and Abnormal Behavior. New York, Marcel Dekker, Inc., in press.

Rosenthal, D.: Was Thomas Wolfe a Borderline? Schizophrenia Bulletin, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00472-15 LPP

PERIOD COVERED

October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

The Investigations of Some Formal Characteristics of Speech

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	T.P. Zahn	Research Psychologist	LPP, NIMH
OTHER:	D.S. Boomer	Guest Worker	LPP, NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Psychology & Psychopathology

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.4	0.3	0.1

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This research project investigates attention during natural speech perception. Specifically, we are attempting to test the hypothesis that speech perception involves phasic, rather than continuous attention, and is characterized by bursts of cognitive activity at linguistically specified boundaries in the stream of speech. The method involves the measurement of reaction time to irrelevant stimuli--clicks--while the subject is listening to a tape recorded dialogue. If the hypothesis is correct, the responses should be relatively inhibited for those clicks timed to occur during the postulated bursts of cognitive activity at linguistic boundaries. If the fundamental hypothesis can be given empirical support this method will be used to investigate attention deficit in schizophrenia.

PHS-6040
(Rev. 10-76)

Project Description:

An extended psycholinguistic research program, detailed in previous annual reports, has contributed to a developing theory of speech production and perception, original in this laboratory. The central hypothesis is that the phonemic clause, a linguistically defined unit of speech averaging about 7 or 8 syllables, is a strategic functional unit in both the production and perception of speech.

During the past year the present research program has focused on speech perception. The guiding hypothesis, growing out of work on listener responses, is that listeners, like speakers, process speech in "chunks," or temporal patterns, the chunks being phonemic clauses. If the decoding process operates with phonemic clause speech units then attention to the incoming speech need not be continuous, even though the speech itself is continuous. Rather than attending to fine detail in the stream of speech, syllable by syllable and word by word, the listener can accumulate a series of 7 or 8 syllables in short-term memory and at its conclusion decode it as a pattern of sound and meaning. If speech perception in fact operates in this way one would expect that the listener's attention would fluctuate, being maximally occupied at the end of the phonemic clause and minimally occupied while the clause is being accumulated and stored.

In the present study subjects listen to a rather absorbing tape recorded dialogue in one ear while also attending to an irregular series of clicks presented to the other ear. They respond to each click by depressing a telegraph key as quickly as possible. Reaction times are measured and stored. Half of the clicks are precisely timed to occur at the end, or terminal juncture, of phonemic clauses; the other half occur within phonemic clauses. Our hypothesis is that the subjects' attention to the cognitive demands of speech decoding will be different during clauses than at the ends of completed clauses, and this difference will be reflected in the mean response latency to the two sets of linguistically-specified clicks.

Results show that for right handed subjects, when speech is presented in the right ear and clicks in the left, reaction times were about 20 msec. faster to clicks at the terminal juncture than to those during the clause (18 of 20 subjects showed this effect). Pilot data on left handed subjects showed no consistent difference. The data suggest that fluctuations of attention do occur during speech perception, but contrary to the original hypothesis speech processing seems to be more active during a clause than at the terminal juncture.

Currently, we are testing the generality of this result by testing a group of left handed subjects and a group of right handers with speech in the left ear and clicks in the right. Laterality is being assessed by means of a questionnaire, behavioral tests and a dichotic listening procedure.

Beyond the present normative studies lie the possibilities of investigating attentional abnormalities or deficits in schizophrenia, with particular attention to the specification of hemispheric asymmetry in cortical function. It is widely believed that cognitive and perceptual deficit in schizophrenia may reflect an underlying disorder of attention, specifically the ability to extract the salient elements of complex stimulus patterns. Since speech is just such a complex pattern and since the hemispheres are asymmetrically specialized for speech, a psycholinguistic approach to the problem of laterality and attention seems to be both neglected and promising.

Publications:

Boomer, D.S.: Speech Units in Human Communication. In Siegman, A.W. and Feldstein, S. (Eds.): Nonverbal Behavior and Communication. Hillsdale, New Jersey, Lawrence Erlbaum Associates, 1978, pp. 245-264.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00473-21 LPP

PERIOD COVERED
October 1, 1977, through September 30, 1978

TITLE OF PROJECT (80 characters or less)

Individual differences in normal perceptual processes.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: V. R. Carlson Research Psychologist LPP NIMH
Other: None

COOPERATING UNITS (if any)

K. L. Norman, Department of Psychology, University of Maryland
I. Feinberg, Veterans Administration Hospital, San Francisco

LAB/BRANCH
Laboratory of Psychology and Psychopathology

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The assessment of perceptual capacities generally requires measurement of the subject's ability to integrate information from more than one stimulus dimension. Judgments of this kind are susceptible to various biases arising from cognitive, motivational, and other context effects, often invalidating the measurement. A general methodology, known as functional measurement theory, is being developed to deal with this problem in behavioral information processing. A study was undertaken to evaluate the usefulness of the functional measurement approach in assessing perceptual constancy judgments. These judgments involve the integration of size and distance information and are known to be affected by attitudinal biases. The question to be investigated was whether the functional measurement procedure would result in valid measurement of perceptual functioning in a situation where specified artifacts and bonafide perceptual effects are known to occur.

Project Description:

The general objectives of this project are to develop valid methods for the assessment of perceptual functioning and to obtain comparison data for evaluating the effects of abnormal and other special conditions. A study was undertaken to investigate the validity of functional measurement theory in assessing perceptual constancy.

Functional measurement theory is a currently developing general methodology for dealing with problems in multidimensional information integration. Perceptual constancy requires the integration of stimulus information such as size, shape, and distance into a unitary percept, and usual measures of the ability to do this are known to be affected greatly by attitudinal biases. A major biasing effect can be demonstrated by varying instructions to subjects to judge apparent visual magnitudes or objective magnitudes. True perceptual interactions, however, occur among different objects when the objects are perceived to be at the same depth, and these interactions become weaker as the objects are perceived to be more greatly separated in space (the adjacency principle). The purpose of the present study was to determine whether reliable and valid estimates of the perceptual effects could be obtained, in spite of the known biasing effects, by applying the techniques of functional measurement.

Functional measurement is a method for evaluating logically and mathematically required consistencies in judgment for different combinations of stimuli. In the present experiment subjects were presented with two triangles and required to adjust the size of a third triangle to the perceived average of the first two. Sixteen size combinations were presented at nine different distance combinations. Normal young adults made the judgments under instructions to match apparent visual size or to match objective, physical size.

The functional measurement analysis was unaffected by attitudinal bias. It correctly indicated a perceptual interaction between two test objects when both were presented at the same near distance. However, it incorrectly indicated the disappearance of the interaction when both objects were presented at the same farther distance rather than when the objects were widely separated in depth.

In studying behavioral effects associated with pathology or drug conditions or in investigating the neurophysiological or biochemical process underlying behavioral effects, it is as essential to achieve scientifically valid measurement of the behavioral variables involved as it is to measure the nonbehavioral variables adequately. Functional measurement theory is potentially wide in scope, applicable in principle to any behavioral assessment of perceptual-cognitive information integration. While superior to more commonly used methods, the present results indicate that the functional measurement approach is nevertheless in need of improvement and further validation.

Publications:

Carlson, V.R., and Feingerg, I.: Perception of the duration of sleep intervals as a function of EEG sleep stage. Physiol. Psychol., in press.

Caron, A.J., Caron, R.F., and Carlson, V.R.: Do infants see objects or retinal images? Shape constancy revisited. Inf. Behav. Develpm., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00476-07 LPP

PERIOD COVERED

October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

The Offspring of Schizophrenics: Markers of a Schizophrenic Disposition

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R.O. Rieder	Research Psychiatrist	LPP, NIMH
OTHER:	D. Rosenthal	Research Psychologist	LPP, NIMH
	S. Broman	Research Psychologist	DNB, NINCDS
	P. Nichols	Research Psychologist	DNB, NINCDS
	P.H. Wender	Professor of Psychiatry	Univ. of Utah

COOPERATING UNITS (if any)

Developmental Neurology Branch, NINCDS

LAB/BRANCH

Laboratory of Psychology and Psychopathology

SECTION

-

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.3	0.2	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This study of children of schizophrenics is based on data from an NINCDS study of the relationship between pregnancy and delivery conditions to psychological and neurological functioning of the offspring, studied until age 8. We have found that: (1) There were more fetal deaths among the offspring of schizophrenics. (2) The IQ scores of the surviving offspring of schizophrenics appear to be more related to adverse perinatal factors than do IQ scores for the controls. (3) The behavior of the surviving male offspring of schizophrenics was more often rated as hyperactive or immature at age 7 than was the behavior of controls. These offspring also had more soft neurological signs, especially abnormal hand-arm coordination.

PHS-6040
(Rev. 10-76)

Project Description:

The project is aimed at discovering differences between the offspring of schizophrenics and controls, at an early stage in life, which may represent a predisposition to develop schizophrenia later in life. It is also aimed at evaluating the effect of perinatal complications on the children of schizophrenics. This study has utilized the data previously collected by the National Institute of Neurological and Communicative Disorders and Stroke, Perinatal Research Branch, in their "Collaborative Study on Cerebral Palsy, Mental Retardation and Other Neurological and Sensory Disorders of Infancy and Childhood." In that project, some 55,000 pregnancies and the offspring were studied, with the 8-year follow-up now just completed. About 900 of the mothers or fathers had had a psychiatric hospitalization, and of this group, we took 220 who were studied in the Boston area to use for our study. We gathered the records of their psychiatric hospitalizations, and we proceeded to diagnose these subjects. We diagnosed 150 parents, and assigned a diagnosis of schizophrenia to 67 of these. The 150 families produced a total of 210 children who had been part of the NINCDS study, and 93 of these were the offspring of schizophrenics. Taking the families containing a schizophrenic parent, we selected a matched control group, and began comparing the findings for our two groups on the investigations which the NINCDS/PRB had performed.

We have found that there is an increased rate of fetal and neonatal death among the offspring of schizophrenics as compared with controls. Some of these deaths were associated with neurological malformations. Reasons for the deaths were not found at autopsy. The deaths were among the offspring of parents with chronic and severe forms of schizophrenia, as opposed to the acute or borderline forms. The deaths occurred predominately among those offspring who had two schizophrenic parents, or had a schizophrenic mother, rather than among those who had a schizophrenic father only. Two comparison groups, offspring of possible schizophrenics and offspring of nonschizophrenic patients, did not show an increased rate of fetal or neonatal deaths when compared to their matched controls.

There is a slightly lower IQ among the offspring of parents with the more chronic forms of schizophrenia when compared with controls at 7 years. Perinatal factors, such as edema, vaginal bleeding, spinal anesthesia at delivery and Apgar Score are quite highly correlated (in a negative direction) with IQ in this sample, whereas they are not highly correlated among the control population. These negative correlations were present whether the father or the mother was the schizophrenic parent, and thus the findings might reflect a genetic susceptibility of the infant to perinatal complications. By means of a multiple regression analysis it was discovered that these perinatal factor-IQ correlations could not be accounted for by demographic variables such as SES, maternal age, parity, etc.

Certain behavioral abnormalities, (hyperactivity, impulsiveness, distractability) were found among the male offspring of parents with the more chronic forms of schizophrenia. We also found a certain combination of abnormalities (hyperactivity and neurological signs), more frequently in these cases than among the controls. It does not necessarily follow that these behavioral disorders are genetically determined, but it is quite possible that the children with these abnormalities are the children who will later become schizophrenic, since hyperactivity is frequently found in the childhood histories of adult male schizophrenics. The perinatal factors which correlated with the offsprings' IQ were not found to correlate highly with these behavioral abnormalities.

The significance of this work to bio-medical research and the program of the Institute is that it adds to our knowledge of the etiology of schizophrenia. We need to find how the genetic factor underlying schizophrenia manifests itself if we are to understand the development of schizophrenia or to effectively intervene.

Publications:

Rieder, R.O., Broman, S.H., Rosenthal, D.: The Offspring of Schizophrenics II. Perinatal Factors and IQ. Arch. Gen. Psychiatry 34: 789-799, July 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00484-18 LPP

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Psychophysiological Responsivity and Behavior in Schizophrenia

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	T.P. Zahn	Research Psychologist	LPP, NIMH
OTHER:	C. Schooler	Research Psychologist	LSES, NIMH
	W.T. Carpenter, Jr.	Director, Maryland Psychiatric Research Center	
	T.H. McGlashan	Staff Psychiatrist	Chestnut Lodge
	M. Buchsbaum	Research Psychiatrist	APB, NIMH
	D. Murphy	Research Psychiatrist	CNB, NIMH
	D. Van Kammen	Research Psychiatrist	BPB, NIMH
	C.R. Lake	Research Psychiatrist	LCS, NIMH
	T.N. Robinson	Staff Fellow	LPP, NIMH

COOPERATING UNITS (if any)

Laboratory of Socio-environmental Studies, Biological Psychiatry Branch, Laboratory of Clinical Science, and Ward 4 East

LAB/BRANCH
Laboratory of Psychology and Psychopathology

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.3	0.7	0.6

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

General purpose: To investigate schizophrenic-normal differences in autonomic nervous system activity in various laboratory situations which differ in the demands placed on the subject, the kinds of stimuli presented and the type of behavior required. To determine biological and psychological processes related to ANS activity.

Peripheral measures of autonomic functioning: Skin conductance, heart rate, finger pulse volume, respiration and skin temperature are recorded continuously during periods of resting, presentation of mild tones and task performance. The tasks used include reaction time, mental arithmetic, two-flash threshold, and tachistoscopic recognition.

We showed that drug free acute schizophrenics who did not improve during a subsequent 4 month hospitalization were more deviant in ANS functioning at admission than were patients who improved. Current studies aim to replicate and extend this finding in patients who are on controlled drug trials to see if on or off drug ANS activity is related to therapeutic outcome.

Project Description:

In a completed study 46 acute schizophrenic patients were tested 3-4 weeks after hospital admission and again 3-4 months later just before discharge, the patients being drug free on both occasions. Compared to 118 normal controls the patients had significantly higher autonomic arousal than the controls on several electrodermal and cardiac indices, but one commonly used index of arousal (skin conductance level) was in the opposite direction. The patients had less phasic ANS responsivity to a series of meaningless tones as well as to the more demanding stimuli of a reaction time task and lower tonic activation during a stress procedure (mental arithmetic test). The schizophrenics also habituated more slowly.

Comparisons of patients who subsequently showed significant improvement at the end of the 4 month hospitalization with patients who were still acutely psychotic showed that despite equally severe psychopathology ratings at the admission testing the admission psychophysiology and attention of the subjects who improved was much more similar to normal than was that of the not-to-improve group. On almost all of the variables which distinguished the patient group as a whole from controls, the not-to-improve group was significantly more deviant from the controls. The tests at discharge showed surprisingly little change from the admission testing even in the patients who showed marked clinical improvement.

These results suggest that early improvement from an acute schizophrenic episode is predictable from ANS activity during the episode but not from the severity of psychopathology during the episode or from conventional prognostic indicators. The small changes in psychophysiology accompanying clinical improvement suggests either that these variables are persisting individual characteristics similar to traits or that "normalizing" of the ANS activity preceded clinical improvement. The patients have been evaluated and retested at a one year followup, and these observations will hopefully clarify the longer-term implications of these findings.

Two new experimental tasks are being used with both schizophrenic patients and normal controls. One is a two-flash threshold determination which purportedly is a measure of "cortical arousal" and which has been found to vary with autonomic arousal level in different directions for schizophrenic and normal subjects. The other is a tachistoscopic recognition procedure which lends itself to concurrent psychophysiological recording. With this technique we hope to be able to test the differential psychophysiological responsiveness of schizophrenics and normals to task difficulty, success and failure, positive and negative reinforcements, etc., as well as being able to separate sensory reception aspects from response aspects of the stimulus complex.

Autonomic functioning has been recorded during performance of these tasks in two sessions which included rest periods and a stress period in which a balloon is blown up until it bursts. These procedures are being given to a

new sample of acute schizophrenic patients during a drug free period shortly after Clinical Center admission. In addition to attempting to replicate the previous findings on the relation of ANS activity to improvement we can also relate the ANS variables to the effects of different drugs such as lithium, pimozide, propanalol, GHB, and naloxone and the effects of renal dialysis which are being tried along with placebo on a double-blind basis on these patients. Thus, we can test the feasibility of using ANS variables to aid in the determination of the optimal therapy. In addition, we are testing patients while they are on the drug protocol using a procedure in which ANS variables are recorded during rest, presentation of a series of tones of different intensities and a two-flash threshold determination. These observations may help us determine how ANS changes relate to changes in behavior and to increase our understanding of the central mechanisms involved in of ANS functioning.

In two studies with normal subjects we are investigating relationships between ANS activity, personality and behavior. Normal subjects (N=97) have been tested on the procedures given to our current schizophrenic group at admission and were given personality, interest, and mood questionnaires and had platelet and plasma monoamine oxidase activity measured. The data relating MAO to personality and interests confirm previous findings that low platelet MAO is related to "stimulus seeking," and suggest that high MAO is related to a rather lethargic approach to life. The large number of psychophysiological variables has been reduced by factor analysis. Scores on factors representing skin conductance responsivity were found to correlate positively with platelet MAO activity for both sexes but most consistently for women. Less consistent positive relationships were found between MAO activity and arousal factors. These data suggest, rather surprisingly, a positive relationship between sympathetic nervous system activity and MAO activity. Other data suggest a negative relationship between MAO activity and parasympathetic activity. Preliminary analyses show that ANS activity is related to low stimulus seeking in women, but to active life style in man. Methods of "confirmatory factor analysis," developed for use in social science research, are being tried on the psychophysiological data in an attempt to further analyze the structure of this system. A similar study emphasizing autonomic orienting responses to tones of different intensities and using an overlapping battery of personality scales is in progress in collaboration with Dr. T. Robinson.

Investigations of the relationships between the ANS response to stress and changes in plasma catecholamines are being done in collaboration with LCS and the NHLBI. Normal controls, hypertensive patients and orthostatic hypotensive patients were given 3 psychological stress procedures (having a balloon popped, mental arithmetic, and electric shocks) and 5 minutes of standing up interspersed with rest periods while skin conductance and heart rate were recorded continuously, blood pressure taken frequently, and periodic blood samples were drawn. These will be analyzed for catecholamines and for dopamine-beta-hydroxylase. Prelimary analyses show hypertensives, who were off medication, were quite low in autonomic response to stress as

indicated by skin conductance and heart rate. A similar study has been completed on 20 middle aged normal males thought either to have a "coronary prone personality" (Type A) or not (Type B). These subjects, selected on the basis of a screening questionnaire, were given a mental arithmetic stress procedure and the "Type A, B" interview as well as the 5 minute standing procedure. To date, plasma norepinephrine has been analyzed in half the subjects. This shows remarkably little increase during psychological stress despite marked autonomic activation, but the expected increase in both sets of measures during standing was found. We hope this study will help elucidate the mechanisms involved in response to stress, will lead to improved methodology for future studies, and will give us some notion of how individual differences in personality relate to physiological responses to stress.

Publications:

Zahn, T.P., and Carpenter, W.T.: Effects of short term outcome and clinical improvement on reaction time in acute schizophrenia. J. Psychiatric Res., in press.

Zahn, T.P.: Comments on "Reaction time and attention in schizophrenia." Schizophrenia Bulletin 3 (3): U.S. DHEW, PHS, ADAMHA, NIMH, DHEW Publ. No. (ADM) 77-145. Washington, D.C., U.S. Govt. Print. Off., 452-456, 1977.

Zahn, T.P., Carpenter, W.T., and McGlashan, T.H. Short term outcome, clinical improvement and reaction time performance in acute schizophrenia. In Wynne, L.C., Cromwell, R., and Matthysse, S. (Eds.): The Nature of Schizophrenia. New York, Wiley, 1978, pp. 273-279.

Schooler, C., Zahn, T.P., Murphy, D., and Buchsbaum, M. Psychological correlates of monoamine oxidase activity in normals. J. Nerv. Ment. Dis. 166: 177-186, 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00486-06 LPP

PERIOD COVERED

October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Psychophysiological Concomitants of Minimal Brain Dysfunction in Children

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	T.P. Zahn	Research Psychologist	LPP, NIMH
OTHER:	P.H. Wender	Professor of Psychiatry	University of Utah
	B.C. Little	Former Guest Worker	LPP, NIMH
	J.L. Rapoport	Medical Officer	BPB, NIMH

COOPERATING UNITS (if any)

Biological Psychiatry Branch

LAB/BRANCH

Laboratory of Psychology and Psychopathology

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	0.2	0.3

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Many investigators believe that the autonomic nervous system may be involved in hyperactivity (HA) in children. The objectives of our studies are to investigate differences in autonomic functioning between HA and normal children by means of peripheral indicators such as skin conductance, heart rate and pupillary activity, to predict which HA will respond favorably to stimulant drugs, and to assess the effects of the drugs on both autonomic activity and on task performance which depends on attention such as reaction time. The hypothesis that HA and normal children respond differently to stimulant drugs has been tested in a study of the effects of d-amphetamine on autonomic activity and attention in 6-13 year-old normal and HA boys. Male adults have been tested on the same protocol to test for age differences in drug effects.

PHS-6040
(Rev. 10-76)

Project Description:

In our previous studies we did not find lower baseline autonomic activation in the HA children when off drugs as some (but by no means all) investigators have claimed. In fact, the HA group had some higher than normal indices of arousal in one session where the amount of environmental stimulation was relatively high. The HA group, however, showed diminished and "sluggish" responsivity to stimuli on several autonomic measures: skin conductance, heart rate, and pupil size. They also were slower in reaction time performance which indicates a deficit in attention. Drugs improved attention and increased baseline activation levels, reduced electrodermal responsivity, and increased anticipatory heart rate deceleration during the reaction time task, a response which is thought to reflect focussed attention. The hypothesis that autonomic variables might predict clinical response to stimulant drugs was not definitely testable because of the very few subjects that failed to respond clinically.

In a current study in collaboration with Dr. J.L. Rapoport of BPB, 6-13 year old normal boys and boys with a diagnosis of HA have been tested on attention (reaction time) and autonomic reactivity (skin conductance and heart rate) after being administered either placebo or d-amphetamine (.5 mg/kg) in a double-blind crossover study. The procedures used are very similar to those used in a previous study with larger samples of HA and normal children. The purpose of this study is to test the widely held hypothesis that HA children give paradoxical or atypical responses to stimulant drugs.

Results show that HA and normal children responded similarly to the drug in terms of reduced motor activity, faster reaction time and increased anticipatory heart rate deceleration. Reduced electrodermal activity was found in both groups but this was more pronounced to different aspects of the test procedure in the two groups being more marked for the normal group in responses to the reaction time stimuli, and in the HA children in responses to simple tones and in spontaneous electrodermal activity. In general, results show that the "paradoxical" quieting and attention-improving effects of stimulant drugs are not specific to hyperactive children, and this conclusion generalizes to psychophysiological effects, at least in part. The difference in patterning of the drug effects on skin conductance responsivity is puzzling. It suggests the changes in behavior may involve different mechanisms in the two groups. Planned correlational analyses may help solve the puzzle.

Normal adult males have been tested on the same protocol and another sample of normal men are being tested on both .5 mg/kg and .25 mg/kg d-amphetamine. Results to date show no drug-related RT improvement in adults.

Publications:

Zahn, T.P., Little, B.A. & Wender, P.H. Pupillary and heart rate reactivity in children with minimal brain dysfunction. J. Abnormal Child Psychology 6: 135-147, 1978..

Rapoport, J. Buchsbaum, M.S., Zahn, T.P., Weingartner, H., Ludlow, C. and Mikkelson, E. Dextroamphetamine: Cognitive and behavioral effects in normal prepubertal boys. Science 199: 560-563, 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00487-06 LPP

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Autonomic Functioning in MZ and DZ Twins

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	T.P. Zahn	Research Psychologist	LPP, NIMH
OTHER:	M. Buchsbaum	Research Psychiatrist	BPB, NIMH
	A. Soskis	Post Doctoral Fellow	BPB, NIMH

COOPERATING UNITS (if any)
Adult Psychiatry Branch

LAB/BRANCH
Laboratory of Psychology and Psychopathology

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The objectives of this project are to determine the relative importance of genetic vs. environmental factors in various aspects of autonomic functioning and to determine the relationships of autonomic nervous system activity to personality, task performance and electrocortical functioning.

Peripheral measures of autonomic functioning, namely skin resistance, heart rate, finger pulse volume, respiration, and skin temperature are recorded during two sessions which include rest periods, a series of mild tones, a reaction time task and mental arithmetic. The subjects are 34 pairs of monozygotic twins and 34 pairs of dizygotic twins, about equally male and female. Some opposite sex DZ pairs have also been tested.

PHS-6040
(Rev. 10-76)

Project Description:

Estimates of genetic influences on ANS variables have been made using the methods of intraclass correlation and comparison of within-pair variances. Significant genetic variance was found for the autonomic "arousal" variables of heart rate, skin conductance level (SCL) and spontaneous skin conductance response (SCR) frequency, for the frequency of SCRs elicited by stimuli, for the rate of recovery of SCRs and for the electrodermal response to stress and for simple reaction time. These findings would seem to have important implications for the study of ANS functioning in psychopathology, since many of these genetically influenced variables discriminate schizophrenics from controls, are related to prognosis within the schizophrenic group, and have found to be related to a predisposition to schizophrenia in subjects with a schizophrenic biological parent.

Further analyses of these data are planned using factor analytic methods to reduce the number of variables and to analyze the structure of the autonomic system. Relationships between the ANS variables and averaged evoked response variables collected on the same subjects by M. Buchsbaum are currently under investigation.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00488-13 LPP

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Individual Differences in Survival and Reproduction Among Old Colony Mennonites

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	G. Allen	Guest Worker	LPP NIMH
OTHER:	C. W. Redekop	Prof. of Sociology and Anthropology	Tabor College

COOPERATING UNITS (if any)

Tabor College

LAB/BRANCH
Laboratory of Psychology and Psychopathology

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This study was undertaken to seek evidence of natural selection with respect to human behavioral characteristics. The population chosen consisted of about 13,000 German-speaking Mennonites whose culture resembles in many respects that of pre-industrial European peasants.

A census-survey was made in 1967 and vital records, kept by the church, were copied. The editing of these two types of record has been very time-consuming, and is still in progress.

When editing is completed, relations will be explored between vital events and socio-economic variables. The principal hypothesis to be tested is that, under conditions of natural human reproduction, individual behavior that is economically successful results also in superior biological reproduction.

PHS-6040
(Rev. 10-76)

Project Description:

This investigation was undertaken in 1965, mainly to seek evidence of natural selection with respect to human behavioral characteristics. The study of selection in a pre-industrial type of European culture may indicate some of the influences that have produced present genetic tendencies and would draw a base line for future studies of selection under modern conditions. A secondary objective was to define natural patterns of marriage and reproduction that might have a bearing on the growth and regulation of human populations.

The population under study consists of about 13,000 German-speaking Mennonites in a politically integrated cluster of 46 villages in northern Mexico. These people were selected because of their simple and uniform culture insulated from mass communication media and because of their apparently unrestricted fertility. Also important was the fact that, unlike some similar religious isolates, this society supports a considerable degree of economic competition.

Professor Calvin Redekop, of the Department of Sociology and Anthropology at Tabor College, has assisted in the planning and execution of the study. He speaks the Low German dialect of these people and has lived among them.

Information was gathered by means of a combined census and survey that reached a third of the population. This was supplemented with photographs of all vital records kept by the church, a public list of farmers and their holdings, a list of all village and church leaders in the 40-year history of the population, and records of Mennonite births in local hospitals.

Editing of the data for accuracy and consistency was the most time-consuming part of the project, because each set of records, even our own census, was collected and recorded by poorly-educated farmers. After editing, the main data sets were subjected to cross-tabulation for general descriptive purposes. The survey data were then analyzed by correlational and factoring methods to yield ratings for each farmer in several dimensions of social and economic behavior. Comparison between the census and church records revealed many disagreements of types which can be reconciled, with resulting enhanced accuracy. This, however, necessitates laborious cross-editing between the two data sets.

The final phase of the study analyzes the relations between vital events and socio-economic variables and attempts to define family characteristics of people who subsequently have large vs. small families.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00489-21 LPP

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Variables Affecting Twin Birth Frequencies

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: G. Allen Guest Worker LPP NIMH

COOPERATING UNITS (if any)

Perinatal Research Branch

LAB/BRANCH
Laboratory of Psychology and Psychopathology

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Significant variations in the twinning rate can be observed both in the long term and in the short term. Both types of variation might be explained under the hypothesis that women who bear dizygotic twins tend to conceive more readily than other women. There are other lines of evidence to support this fertility hypothesis.

Data on about 50,000 pregnancies collected prospectively in the Collaborative Perinatal Project permitted a direct test of the hypothesis and excluded it.

Project Description:

This project constituted the final phase in a program of research begun in 1957. The earlier research led to the hypothesis that women who bear dizygotic twins are particularly fertile, a hypothesis reached independently by Finnish geneticists and supported by data from several sources. From U.S. birth statistics I was able to estimate the apparent difference in waiting time to conception, called "fecundability," between twin-prone women and other women.

If twin-prone women are more fertile than others, this fact, interacting with contraception, might explain the known variation in twin birth frequencies among countries and between years or decades.

Nearly ideal material for testing the fertility hypothesis was collected in the Collaborative Perinatal Project. The Perinatal Research Branch, NINCDS, extracted from their file the relevant information on about 50,000 pregnancies, and this condensed file was used in the present analysis.

Findings:

Although mothers of DX twins did, as predicted, have more births at every stage of their reproductive life than mothers without twins, there is another possible explanation besides the fertility hypothesis. When women are matched for age, the likelihood of having twins may increase with parity. This second explanation was confirmed by the following finding: Among births to mothers of twins in any five-year maternal age group, twin births occurred at a higher average parity than did singleton births. Since this effect was sufficient to account for all the difference in birth order, it appears that mothers of twins are not more fertile than other women, and so the variations in twinning rate are not susceptible to the most parsimonious explanation.

Publications:

Allen, G.: The Parity Effect and Fertility in Mothers of Twins. In Nance, W.E., Allen, G., and Parisi, P. (Eds.): Twin Research. New York, Alan R. Liss, Inc., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 MH 00491-02 LPP

PERIOD COVERED
October 1, 1977, through September 30, 1978

TITLE OF PROJECT (80 characters or less)
Personality factors and psychophysiological response to changing stimulus input.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	T. N. Robinson, Jr.	Staff Fellow	LPP NIMH
Other:	T. P. Zahn	Research Psychologist	LPP NIMH
	R. O. Rieder	Research Psychiatrist	LPP NIMH

COOPERATING UNITS (if any)
None

LAB/BRANCH
Laboratory of Psychology and Psychopathology

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.0	2.5	.5

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The project is designed to shed information on how such factors as stimulus intensity, unchanged stimulus input, heteromodal stimulation, and sensory threshold task performance differentially affect autonomic nervous system (ANS) functioning in selected personality groups. Examined also is the relative correspondence of bilateral electrodermal activity to perceptual task performance. The relation of psychiatric diagnosis to electrodermal responsivity is examined by recalling a selected group of previously-tested subjects and providing them with a psychiatric interview. Black and white males are sampled in an effort to provide data on racial differences in ANS functioning to imperative and uncontrolled stimulus input.

PHS-6040
(Rev. 10-76)

Project Description:

The objectives of this project are to provide further clarification of the relationship of psychometric personality factors and psychophysiological response in normals and to assess the extent to which racial groups show ANS response stereotypy.

Data collection for each subject comprises two sessions separated by at least a week. Each session involves approximately 2½ hours of testing. Ninety volunteers have been tested to this point.

Preliminary findings for the first session data indicate that personality differentially affects the pattern of electrodermal arousal under mildly stressful stimulation conditions. Significant differences in auditory threshold performance have also been demonstrated for a number of personality measures.

This project is significant to biomedical research and the program of the Institute in that a greater clarification of ANS functioning in normals will make more meaningful the comparison of normals and psychiatric patients in future psychiatric research. Moreover, because personality structure may play an important role in the expression of psychopathological illness, it is possible that a determination of the patterns of psychophysiological response associated with personality factors may help account for the large variability in ANS response noted among psychiatric patients. The determination of differential ANS response patterns for racial groups has implications for the genetics of psychophysiology and, as such, for the use of these techniques in discriminating aspects of the genetical component of mental illness.

The proposed course of the project is to continue testing until an additional 60 subjects have been tested. The project has been expanded recently to include psychiatric interview information on selected individuals previously tested under the original protocol.

Publications:

Robinson, T.N., Jr., Rosenthal, D., and Rasmussen P.V.: Spectrum diagnosis and ego strength functioning as related to electrodermal activity in a conditioning paradigm. J. psychiat. Res. 13: 257-272, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00492-02 LPP

PERIOD COVERED: October 1, 1977, through September 30, 1978

TITLE OF PROJECT (80 characters or less)

Relationships among personality, biochemical and psychophysiological variables in college students.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. J. Haier	Staff Fellow	LPP	NIMH
Other:	M. S. Buchsbaum	Chief, Psychophysiology Section	BPB	NIMH
	D. L. Murphy	Chief, Clinical Neuropharmacology	LCS	NIMH

COOPERATING UNITS (if any)

LAB/BRANCH: Laboratory of Psychology and Psychopathology

SECTION:

INSTITUTE AND LOCATION: NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	.5	.5

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Monoamine oxidase (MAO) activity and the average evoked response measure of augmenting/reducing (AR) differentiate some psychiatric patient groups from normal controls. This study tested whether a combination of MAO and AR predict psychiatric vulnerability in a nonhospitalized and previously undiagnosed sample. Almost 400 college males were screened on the Minnesota Multiphasic Personality Inventory (MMPI). A subsample of students with indications of psychological problems was selected as was a control sample. These students were classified into 4 biologically homogeneous groups--low MAO/augmenting, low MAO/reducing; high MAO/augmenting, high MAO/reducing. Based on the Research Diagnostic Criteria (RDC), more psychopathology is found among low MAO/augmenting and high MAO/reducing students. This result replicates the pattern indicated in a previous study and suggests that specific biological variables may be related to a general vulnerability to psychiatric problems.

PHS-6040
(Rev. 10-76)

Project Description:

This project was designed to identify people who have experienced psychological difficulties or psychiatric symptoms but who have never been hospitalized, treated, or diagnosed and then to test whether there are biological parallels between such cases and psychiatric inpatients.

To generate the sample, 385 college males were screened on the Minnesota Multiphasic Personality Inventory (MMPI). Everyone with aberrant scores and a control sample with normal scores also completed monoamine oxidase (MAO) testing, average evoked response (AER) testing, and a structured interview with a psychiatrist using the Research Diagnostic Criteria (RDC).

Eighty-two percent of the cases with aberrant MMPI scores met the RDC for at least one diagnosis, whereas only 22% of the controls met any RDC. All subjects were classified into 4 biologically homogeneous groups--low MAO/augmenting, low MAO/reducing, high MAO/augmenting, and high MAO/reducing. The frequency of RDC diagnoses in each biological group showed that most psychopathology was found among low MAO/augmenters and high MAO/reducers. A similar pattern was noted among psychiatric patients.

We conclude that a research sample identified by MMPI and RDC screening of a nonhospitalized group yields many individuals with some psychopathology and that these individuals show a specific biological parallel to psychiatric patients.

The proposed course of the project is to follow-up the student subjects to determine whether the biological groups predict risk for future psychiatric problems.

This project is significant to the Institute's research program because it may have implications for diagnosing psychiatric disorders using biological variables and for population screening studies of mental health.

Publications:

Haier, R.J., Buchsbaum, M.S., and Murphy, D.L.: Screening young adults for psychiatric vulnerability: A preliminary comparison of biological and clinical measures. Psychopharm. Bull., in press.

Haier, R.J., Rieder, R.O., Khouri, P.J., and Buchsbaum, M.S.: Extreme MMPI Scores and the Research Diagnostic Criteria: Screening college males for psychopathology. Arch. gen. Psychiatry, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00493-02 LPP

PERIOD COVERED

October 1, 1977, through September 30, 1978

TITLE OF PROJECT (80 characters or less)

Studies of thought disturbance

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. J. Haier	Staff Fellow	LPP NIMH
OTHER:	H. Weingartner	Acting Chief	LPP NIMH

COOPERATING UNITS (if any)

Ward 4E, BPB, NIMH

LAB/BRANCH

Laboratory of Psychology and Psychopathology

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project has been inactive during this report period.

PHS-6040
(Rev. 10-76)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00494-02 LPP

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Clinical and Empirical Detection of Simulated Schizophrenia

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R.O. Rieder	Research Psychiatrist	LPP, NIMH
OTHER:	D.Rosenthal	Research Psychologist	LPP, NIMH
	R. Haier	Research Psychologist	LPP, NIMH
	Paul Alexander	Assistant Professor of Psychiatry	Brown Univ.
	Victor Reus	Clinical Associate	BPB, NIMH

COOPERATING UNITS (if any)

St. Elizabeth's Hospital, Psychodrama Section

LAB/BRANCH
Laboratory of Psychology and Psychopathology

SECTION

INSTITUTE AND LOCATION
ADAMHA, NIMH, NIH, Bethesda, MD 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.3	0.1	0.2

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☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project explores the ability of psychiatrists and others in differentiating actors simulating schizophrenia from schizophrenic patients, using a 30 minute interview. The initial stage of the project has been completed. Twenty-seven subjects (15 "actors" and 12 patients) were interviewed by an NIMH psychiatrist, and he identified 24 of the 27 subjects correctly. Videotapes were made of the interviews and segments of the tapes have been shown to audiences composed of psychiatrists and other raters. The ratings, based on the videotapes, are now being analysed.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00495-02 LPP

PERIOD COVERED
October 1, 1977 through September 30, 1978

TITLE OF PROJECT (80 characters or less)
The storage and retrieval of experience: psychological and biological determinants.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	H. Weingartner	Acting Chief	LPP NIMH
OTHER:	E. D. Caine	Medical Officer	LCS NIMH
	M. H. Ebert	Chief, Sec. Exper. Therapeutics	LCS NIMH
	P. Gold	Chief, Unit on Neuroendocrinology	CPB NIMH
	R. Haier	Research Psychologist	LPP NIMH
	D. L. Murphy	Chief, Neuropharmacology Branch	CNB NIMH
	R. W. Post	Chief, Section on Psychobiology	BPB NIMH
	J. L. Rapoport	Medical Officer	BPB NIMH
	V. Reus	Clinical Associate	BPB NIMH
	D. P. van Kammen	Chief, Sec. on Neuropsychopharmacology	BPB NIMH
	A. Zis	Guest Worker	CPB NIMH

COOPERATING UNITS (if any)
Biological Psychiatry Branch, DCBR, NIMH
Laboratory of Clinical Science, DCBR, NIMH
Clinical Neuropharmacology Branch, DCBR, NIMH

LAB/BRANCH
Laboratory of Psychology and Psychopathology

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.8	0.2

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☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The research efforts described here have been designed to explore cognitive processes in man. The studies have been constructed to bridge aspects of the psychology and biology of thinking, learning, and remembering. To accomplish this, studies have explored 1) disturbances in cognition as they appear in depression, mania, schizophrenia, the hyperactivity syndrome in children, the organic dementias; 2) characteristics of changes in cognitive processes as these occur in response to potential therapeutic-psychoactive drugs in patient groups; 3) studies of drug-induced changes in cognition in normal controls; 4) measurement and manipulations of behaviors that are related to cognitive functioning in psychiatric patients as well as in normal controls.

Project Description:

This research has been designed to explore the underlying biological-psychological mechanisms of cognitive processes with a particular focus on separating and analyzing separate components and factors that define the storage and retrieval of information. These include attentional determinants, short-term memory, long-term memory, memory-trace consolidation, encoding strategies, retrieval strategies, organization in memory, and biological-psychological determinants that link each of these components. These same research efforts, designed to explore normal cognitive mechanisms, have also been refined and designed for use in uncovering some of the cognitive structure of a variety of psychiatric disorders. Since much of disturbed behavior is reflected in how patients think about, experience, learn, and remember features of the world around them, an understanding of the unique cognitive processes that occur in these disruptions or changes in psychiatric illnesses is particularly important in understanding the ethology, prognosis, and potential treatments of these disorders.

Research studies have been developed around the following interrelated foci:

1. Mechanisms and strategies that alter the effectiveness of learning-memory and cognition. The studies have involved:

   a) Manipulations of cholinergic mechanisms in patients and normal controls.
   b) Drug studies in normal adults and depressed patients, as well as normal and hyperactive children.
   c) Studies of stimulus characteristics and retrieval determinants in defining memory storage, retrieval, and encoding processes.
   d) Cognitive studies involving demented patients both under drug and drug-free conditions.

2. State-dependent learning. This area of interest continues to provide a useful framework for exploring the following:

   a) The qualitatively unique manner in which events are stored (encoded) and retrieved from memory.
   b) Studies of disturbances in mood state.
   c) Qualitative changes in cognition in response to psychoactive drugs.
   d) Contextual factors as determinants for defining the nature of trace events in memory.

3. Studies of encoding processes and memory consolidation. This research has focused on:

   a) Stimulus characteristics as determinants of encoding.
   b) Arousal-activation in modulating the consolidation (long-term storage) of trace events in memory.
   c) Neurochemical correlates of different components of memory-trace formation and retrieval.
   d) Development of more effective techniques for defining the strength of memory traces.

4. Studies of the dementias in man. These involve investigations which compare and contrast different forms of organic dementias, including Alzheimer's Disease, Huntington's Disease, Korsakoff Psychosis, etc. Studies are designed to explore in detail the specific manner in which cognition is disrupted, altered by drugs, and implications for defining normal cognition. These studies have explored:

   a) Encoding factors, depth of processing.
   b) Retrieval consistency.
   c) Central nervous system response to psychoactive drugs and related cognitive responses.

5. Studies of cognition in the mood disturbances.

   a) Cerebral lateralization of cognitive functions in depression and mania.
   b) Disturbances in arousal/activation as determinants of disruptions in memory consolidation in depression.
   c) State-dependent learning in depression and mania.

6. Psychological-biological determinants in the cognition of children.

   a) Studies of components of drug-altered and drug-free cognition in normal children.
   b) Studies of cognition in hyperactive children.
   c) Studies of learning disabiltiies in children.

Methods

The studies outlined above have been carried out using a large variety of learning procedures which have been specifically designed and developed by this investigator to explore focal issues that might relate cognition to specific aspects of brain state. The research questions have necessarily required the construct of some specific instruments rather than the use of "standard" psychological tests. These include:

1) Strategies for manipulating the encoding of events. These techniques include the use of sorting procedures and accompanying uncertainty analyses (Caine, Ebert, & Weingartner, 1978; Weingartner, Caine, & Ebert, in press; Weingartner, Docherty, & van Kammen, in press); manipulation of context at the time of storage and retrieval (Weingartner, 1978; Weingartner & Murphy, 1977); systematic changes in the kinds of stimuli to be processed and their encodability (Weingartner, Adefris, Murphy & Eich, 1976; Weingartner & Murphy, in press); changes in the surrounding consequences or context for storing and retrieving events (Weingartner, Martello, & Murphy, in press).

2) Immediate and delayed recall as well as analysis of input serial position in relation to output of information at the time of retrieval. These

techniques have been used to explore stage models of information processing and retrieval, as well as consolidation theories of learning.

3) A "levels of processing" approach to information storage and retrieval. The techniques embodied in this approach use methods for manipulating depth of salience of processing (through manipulations of arousal or orienting task) as a means of defining how effectively input information has been encoded. It also stresses distinctions in retrieval analysis between "availability" and "accessibility" of learned events.

4) Free recall, serial learning, prompted recall, paired associates, cued recall, recognition memory, pattern learning as well as other procedures, have been designed for use in examining specific hypotheses that might capture characteristics of processes.

5) Techniques for measuring consistency in recall as a means of determining the strength of memory traces. Some of these tools also involve means for measuring characteristics of memory consolidation.

Major Findings

1. Mechanisms and strategies that define learning-memory encoding and memory-trace consolidation.

   a) Drugs, such as amphetamine, affect (enhance) some components of memory-trace formation in normal children (Rapoport, Buchsbaum, Zahn, Weingartner, Ludlow, & Mikkelsen, 1978; Weingartner, Rapoport, Buchsbaum, Ebert, Caine, & Mikkelsen, 1978); in hyperactive children (Weingartner & Rapoport, 1978); and in normal adults. The response to amphetamine in these populations also highlights characteristics of encoding, trace consolidation, and depth of information processing.
   b) Physostigmine, arecholine, and choline chloride affect different components of information storage and retrieval from those affected by amphetamine treatment (Sitaram, Weingartner, Caine, & Ebert, 1978; Sitaram, Weingartner, & Gillin, in press; Weingartner, Sitaram, & Gillin, in press). Dopaminergic vs. cholinergic factors appear to play different roles in affecting the storage, consolidation, and retrieval of information.
   c) Specific changes in learning and memory are related to changes in neurochemical activity (Weingartner, Rapoport, Buchsbaum, Ebert, Caine, & Mikkelsen, in press).
   d) Stimulus factors such as imagery, processing mode, speed of processing, emotional impact, and arousal factors alter the strength of trace learning but not necessarily how much is learned (Weingartner, Caine, & Ebert, in press; Weingartner & Caine, in press; Weingartner & Caine, in press; Caine & Weingartner, in press).

2. State-dependent learning (SDL), state-dependent storage (SDS), and state-dependent retrieval (SDR).

   a) Changes in the cholinergic nervous system induce state-dependent storage (SDS) and state-dependent retrieval (SDR) of information. This is evident in response to physostigmine, arecholine, choline chloride, and scopalomine. (Weingartner, Sitaram, Gillin, & Murphy, in press).
   b) Changes in brain state, post-processing, are capable of inducing the formation of qualitatively unique trace events, SDL, SDS, and SDR (Weingartner, Sitaram, Gillin, Murphy, & Redmond, in press).
   c) SDL-SDS-SDR accounts for many of the clinical phenomena that define the uniqueness of experience and remembering in different psychiatric disorders (Reus, Weingartner, & Post, in press).
   d) Mood represents a context for encoding events and generating retrieval strategies for remembering (Weingartner, Murphy, & Stillman, in press).
   e) Self-generated events are more susceptible to dissociation in memory, and this relates to the episodic nature of how experiences are stored in memory (Weingartner, Murphy, & Stillman, in press; Eich, Weingartner, & Stillman, in press).

Clinical Studies and Implications for Models of Memory and Learning.

1. Depressed patients demonstrate disruptions in learning and memory. These are qualitatively different from the pattern of cognitive changes seen in other psychiatric disorders (Cohen, Murphy, & Weingartner, 1978).

2. In response to amphetamine, learning and memory are enhanced in depression, and this effect is most evident in the formation of salient memory traces rather than how much is learned (Reus, Post, & Weingartner, in press).

3. Patients with histories of mood disturbance, when treated with lithium, demonstrate disruptions in information storage which are lithium-response specific (Reus, Post, Weingartner, in press).

4. We have explored the use of behavioral and pharmacological tools for altering the cognitive pathology in some of the dementias. This work is still in a preliminary stage: Findings related to encoding-processing changes that mark the cognitive disruptions in some syndromes seem to be a particularly relevant system that determines the disruption in cognition in these syndromes (Caine & Weingartner; Weingartner & Caine; Ebert, Sitaram, & Weingartner; submitted for publication).

5. Studies of drug-treated (amphetamine) and untreated hyperactive children have uncovered some of the basic components of cognitive processing that are disrupted in these children, altered by stimulant-drug treatment, and left unaffected by drug treatment in the absence of psychological treatments (Weingartner & Rapoport, APA, 1978; Weingartner, Rapoport, Buchsbaum, Ebert, Caine, & Mikkelsen, in press).

Significance to Mental Health Research: The use of cognitive models has provided a powerful framework within which it is possible to describe important characteristics of schizophrenic disorders, depression, mania, organic dementias, and drug-altered states in patients and normal controls. This research effort has proved to be a particularly useful tool in relating biological and psychological phenomena as these are expressed in these different disorders. The clinical findings have also been useful in building more viable theories of how information is stored, learned, and retrieved and of the central nervous system determinants and representations of these behavioral events. The studies are seen as useful in better understanding 1) the etiology and expression of a variety of psychiatric syndromes, 2) exploring tools that are therapeutically useful; 3) evaluating therapeutic strategies; and 4) allowing for a better understanding of normal cognitive processes.

Proposed Course of Projects: These research efforts have been quite productive. Based on previous findings and approaches, future research efforts will be directed at 1) further defining interrelated biological-psychological determinants of cognitive processes, particularly those involved in encoding and consolidation; 2) development of a more effective framework for defining disturbances in cognition and changes in cognition in response to psychoactive drugs; and 3) for the first time, we are in a position not only to define and characterize some of the biology and psychology of normal and disordered cognition but will be able to enhance and alter a number of disturbances in cognitive functions. These efforts will include pharmacological treatments in a number of clinical populations (depression, Alzheimer's Disease) as well as psychological techniques for manipulating cognitive strategies, e.g., in learning-disabled children.

Dissemination of Information:

I. American Psychiatric Association Annual Meeting, Atlanta, Ga., May, 1978.

   A. Co-chaired special session.
   B. Papers presented:
      1. Cognitive perspectives for the study of neuropsychiatric disease: Classifying cognitive disorders.
      2. Current models of learning and memory.
      3. Classification of cognitive disorders (with Eric Caine).

II. Biological Psychiatry Meeting, Atlanta, Ga., May, 1978 - Paper presented (with Reus): D-Amphetamine: Effects on memory in a depressed population.

III. First International Symposium on Drugs as Discriminative Stimuli, Beerse, Belgium, July, 1978 - Papers presented:

   A. Physostigmine state-dependent learning in man.
   B. Mood state dependent learning.

IV. American Psychological Convention, Toronto, Canada, August 28 to September 1, 1978.

A. Chaired symposium.
B. Papers presented:

1. Psychopharmacological approaches to the study of cognitive mechanisms.
2. Lateralized cognitive changes in depression.
3. Special cognitive effects of amphetamine in normal and hyperactive children.

Publications:

Weingartner, H., Miller, H.A., and Murphy, D.L.: Affect state-dependent learning. J. Abnormal Psychol. 86: 276-284, 1977.

Weingartner, H. and Murphy, D.L.: State-dependent storage and retrieval of experience. In Birnbaum, I.M. and Parker, E.S. (Eds.): Alcohol and Human Memory. New York, John Wiley and Sons, 1977, pp. 159-176.

Weingartner, H., Murphy, D., and Stillman, R.C.: Drug and Mood state specific encoding and retrieval of experience. In International Challenge of Drug Abuse, NIDA Monograph 19, DHEW/Pub. ADM-78-654, 1978, pp. 282-297.

Weingartner, H.: Human state-dependent learning. In Ho, B.T., Richards, D.W., and Chute, D.C. (Eds.): Drug Discrimination and State Dependent Learning. New York, Academic Press, 1978, pp. 361-382.

Caine, E.D., Ebert, M.H., and Weingartner, H.: An outline for the analysis of dementia: The memory disorder of Huntington's Disease. Neurology 27(11): 1087-1092, Nov. 1977.

Caine, E.D., Hunt, R.D., Weingartner, H., and Ebert, M.H.: Huntington's Dementia: A review of clinical and neuropsychological features. Arch. of Gen. Psychiatry 35: 377-384, March 1978.

Rapoport, J., Buchsbaum, M., Zahn, T., Weingartner, H., Ludlow, C., and Mikkelsen, E.: Dextroamphetamine: Cognitive and behavioral effects in normal prepubertal boys. Science 199: 560-562, 1978.

Sitaram, N., Weingartner, H., and Gillin, J.C.: Choline: Selective enhancement of serial learning and encoding of low imagery words in man. Life Sciences 22: 1555-1560, 1978.

Stillman, R.C., Welkowitz, O., Weingartner, H., DeRenzo, E.V., and Wyatt, R.F.: Marijuana: Differential effects on right and left hemisphere functions in man. Life Sciences 21(12): 1793-1800, 1977.

In Press:

Weingartner, H. and Murphy, D.L.: Amphetamine state dependent learning. Journal of Abnormal Psychology.

Weingartner, H. and Caine, E.D.: Cognitive perspectives for the study of neuropsychiatric disease: I. Current theories of learning and memory. American Journal of Psychiatry.

Weingartner, H., Caine, E.D., and Ebert, M.H.: Experimental psychological approaches to a human memory disorder: Information storage and retrieval processes in Huntington's Disease. Cognitive Psychology.

Weingartner, H., Caine, E.D., and Ebert, M.H.: Imagery, encoding and the retrieval of information from memory: Some specific encoding-retrieval changes in Huntington's Disease. Journal of Abnormal Psychology.

Weingartner, H., Murphy, D.L., Stillman, R.C.: Mood state dependent learning. First International Symposium on Drugs as Discriminative Stimuli Journal.

Weingartner, H., Murphy, D.L., and Martello, J.: Mood state dependent learning following the experience of success or failure. Journal of Consulting and Clinical Psychology.

Weingartner, H., Sitaram, N., Gillin, J.C., Murphy, D.L., and Eich, J.: Physostigmine state-dependent learning in man. First International Symposium on Drugs as Discriminative Stimuli Journal.

Weingartner, H., Van Kammen, D.L., and Docherty, J.: The schizophrenic thought process: Structure, storage and retrieval of information from memory. Journal of Abnormal Psychology.

Weingartner, H., Martello, J., and Murphy, D.L.: The structure of information storage and retrieval in the depressed state. British Journal of Psychiatry.

Caine, E.D. and Weingartner, H.: Cognitive perspectives for the study of neuropsychiatric disease: II. Classifying cognitive disorders. American Journal of Psychiatry.

Eich, J.E., Weingartner, H., Stillman, R.C., and Wyatt, R.: State-dependent retrieval of item associative and serial-order information. First International Symposium on Drugs as Discriminative Stimuli Journal.

Reus, V.I., Weingartner, H., and Post, R.M.: Clinical implications of state dependent learning. American Journal of Psychiatry.

Reus, V.I., Targum, S.D., Weingartner, H., and Post, R.M.: Effect of lithium carbonate on memory processes of bipolar affectively ill patients. Psychopharmacology.

Shoulson, Il, Caine, E.D., Falin, S., Kobayashi, R., Kohmen, E., Sac. D., Weingartner, H., and Wexler, N.: Clinical care of the patient and family with Huntington's Disease. Commission for the Control of Huntington's Disease.

Sitaram, N., Weingartner, H., and Gillin, J.C.: Human serial learning: Enhancement with arecholine and choline and impairment with scopolamine correlate with performance on placebo. Science.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00496-02 LPP

PERIOD COVERED

October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Animal Models of Psychopathology

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: S.M. Anderson Staff Fellow LPP, NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Psychology and Psychopathology

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, MD 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The goal of my research is to gain a better understanding of human psychopathology through the study of biochemical and behavioral variation in lower animals. Presently this objective is being pursued through a study of normal variation in levels of monoamine oxidase (MAO) activity in the laboratory mouse. Brain MAO activity was measured in the brains of female and male animals of a genetically heterogeneous strain at 100, 200, 300 and 400 days old using PEA, 5-HT and tyramine as substrates. Brain MAO activity remained constant across all age groups when PEA and 5-HT were used as substrates but a sharp decrease in tyramine deamination was observed in the brains of 400 day old subjects. This variation in activity may be due to deterioration of the enzyme binding site, loss of tyramine affinity due to inhibition or competition from increases in endogenous substances in the brain with aging. Different levels of MAO activity has been demonstrated in the brain homogenates from selected inbred strains of mice using PEA, 5-HT and tyramine as substrates. The possibility of a classical Mendelian pattern of inheritance is being studied in F_1, F_2 and backcross generations using inbred lines selected from the previous study.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00497-02 LPP

PERIOD COVERED

October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

The development of an instrument that delineates the syndrome of borderline schizophrenia.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Philippe Khouri, M.D. Research Psychiatrist LPP, NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Psychology and Psychopathology

SECTION

-

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Md. 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1	1	

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project has been discontinued.

PHS-6040
(Rev. 10-76)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00498-02 LPP

PERIOD COVERED

October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Personality assessment of the first-degree relatives of schizophrenics.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Philippe Khouri, M.D.	Research Psychiatrist	LPP,NIMH
OTHER:	Richard Haier, Ph.D.	Research Psychologist	LPP,NIMH
	David Rosenthal, Ph.D.	Research Psychologist	LPP,NIMH

COOPERATING UNITS (if any)

Adult Psychiatry Branch

LAB/BRANCH

Laboratory of Psychology & Psychopathology

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Md. 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1	1	

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☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

First-degree relatives of schizophrenics have been described in the clinical literature as having certain personality abnormalities like "introversion, suspiciousness, lacking in interpersonal effectiveness..." This study selects 60 first-degree relatives of schizophrenics and a control group of 60 first-degree relatives of manic-depressives. The Research Diagnostic Criteria (RDC) system is used to select the two groups. Three personality tests, the California Psychological Inventory (CPI), the Myers Briggs and the Cattell 16PF are used to assess the personality characteristics that may be specific to individuals biologically related to a group of schizophrenics.

PHS-6040
(Rev. 10-76)

Project Description

Ever since E. Kraepelin and E. Bleuler reported that some relatives of schizophrenics have certain kinds of peculiarities in their personalities variously described as "eccentric, irritable, introvert, lacking in interpersonal effectiveness, suspicious, odd ...," many reports about the psychological characteristics of relatives of schizophrenics have appeared in the literature. Some reports are anecdotal, like those of Kraepelin and Bleuler, while others used projective tests like the Rorschach or objective type of tests like the MMPI.

Singer and Wynne were able to discriminate the parents of schizophrenics from those of neurotic and normal subjects, using the Rorschach. Other investigators, however, were unable to confirm their findings.

A study by Cohler, et al., on the parents of schizophrenics, neurotics, "borderline personalities" and normals revealed no difference in the MMPI profiles of the four groups.

There are two possible explanations for these negative results:

1. The number of relatives of each group was too small to show any significant difference between the MMPI group profiles.
2. The MMPI is standardized against clinically sick and hospitalized populations and may not be suitable for use in a population where most members are not hospitalized for gross psychopathology as in the relatives of mental patients in general.

In the proposed study we will use objective personality tests that are standardized against non-clinical populations but measure personality characteristics like introversion/extraversion, interpersonal effectiveness, suspiciousness/trust, sociability, etc.

The three tests we will use are: The California Psychological Inventory (empirically derived), The Myer-Briggs (rationally derived) and the Catell 16PF (factor-analytically derived).

If significant differences are found among the first-degree relatives of both groups, a sample of subjects from both groups with controls from within each group will be recalled and given a semi-structured psychiatric interview with the CAPPS. This would allow us to correlate certain personality traits with clinical psychopathology.

Twenty patients from the Clinical Center and St. Elizabeth's Hospital, with the diagnosis of schizophrenia, and 20 patients with the diagnosis of manic disorder will be selected. The probands selected will either be hospitalized or former patients of the wards. However, their inclusion in the study will be done in coordination with the clinical staff or the therapists seeing them. Each patient's diagnosis will be checked against the Research Diagnostic Criteria of Spitzer, Endicott, and Robins, and each patient

should have first-degree relatives in the Washington area. With the knowledge of the patient, all his/her first-degree relatives (over the age of 15) in the Washington area will be contacted (first by mail, then by phone) and asked to come to the National Institutes of Health in Bethesda, for a three-hour session. During this session the three personality tests will be administered. The tests may be given individually or in groups; all relatives completing the session will receive standard payment. We expect to test an average of 3 relatives per patient. The patient also will be asked to complete the same tests under the supervision of ward personnel (the items on all three tests are very similar to MMPI items, and should present no particular problem

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00499-01 LPP

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Biological and Family Studies of Chronic Schizophrenia

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: R.O. Rieder Research Psychiatrist LPP, NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Psychology and Psychopathology

SECTION

INSTITUTE AND LOCATION
ADAMHA, NIMH, NIH, Bethesda, MD 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.7	0.5	0.2

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project aims to determine if certain biological factors, namely low platelet MAO, abnormalities on the CAT Scan, or left-handedness have an etiological relationship with schizophrenia. The method involves finding schizophrenics with one of these factors and studying the relatives to see if the factor and schizophrenia segregate together in these pedigrees.

The work on this project to date has involved only sample collection (i.e., interviewing schizophrenics) and the development of an interview schedule for borderline schizophrenia. No analyses have yet been done.

PHS-6040
(Rev. 10-76)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00671-09 LSES

PERIOD COVERED
October 1, 1977, to September 30, 1978

TITLE OF PROJECT (80 characters or less)
Social Origins of Stress

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Leonard I. Pearlin	Research Sociologist	LSES NIMH
Other:	Elizabeth Menaghan	Psychologist	LSES NIMH

COOPERATING UNITS (if any)
None

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Laboratory of Socio-environmental Studies

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3	2	1

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☐ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The research examines the relationship between social experience and psychological well-being. It looks at the enduring problems people encounter in their daily lives as workers and family members, at the gain and loss of roles as the life-cycle unfolds, and at sudden, unexpected crises that often confront people. Each of these types of experience is examined in relation to symptoms of emotional distress. Data for the study were collected at two points in time. In 1972 a sample of 2300 people, representative of the Chicago Urbanized Area were interviewed, and a sub-sample (1200) of this group was reinterviewed in 1976-77. Analyses have been made to identify the events that are particularly likely to result in emotional distress, and to understand how people use psychological and social resources to cope with the problems and vicissitudes of life.

PHS-6040
(Rev. 10-76)

Project Description:

This is a longitudinal study of social stress whose data were collected at two points in time, spanning a period of about four years. In 1972 interviews were conducted with a representative cluster sample of 2300 adults in the urbanized area of Chicago. These interviews sought information about three features of people's lives: 1)the chronic, often low-keyed, everyday problems that people encounter in their occupational, marital and parental roles; 2)the coping strategies people employ in dealing with life-problems; and 3)symptoms of emotional stress, anxiety and depression. Then in 1976-77 a second wave of interviews was conducted among a sub-sample of 1200 people drawn from those originally interviewed. Many of the questions initially asked about the chronic role problems, coping, and psychological distress were repeated, thus providing longitudinal information about stability and change on these dimensions of people's lives. The second interview, in addition, sought information about potentially distressful types of life-events that were not included in the 1972 interviews. One type is represented in the many changes and role transitions that are normative aspects of the life-cycle--e.g., being married, having a child, retiring from work, etc. A second type of threatening event is represented in the more unexpected, non-normative disruptions of important roles and activities, such as occur with divorce, serious illness, involuntary unemployment, and other life crises.

Together the two interviews provide a large body of information about problems woven into the fabric of major social roles, the gains and losses involved in life-cycle transitions, the crises that can eruptively intrude into people's lives, coping repertoires and psychological outcomes. It is the long-range goal of the research to reconstruct from this information those elements of social experience that importantly affect mental health and to learn how people attempt to avoid being psychologically harmed by such experience.

Much of the work of the past year has proceeded along methodological lines. In examining our longitudinal data, it became clear that traditional methodologies for assessing change were too unreliable for our needs. We require rather precise indicators of changes in emotional distress in order to evaluate accurately the impact of social experiences and life events on psychological functioning. Existing measures of psychological functioning contain error, as do most social and psychological measures. There is now a substantial literature showing that the distortions introduced by even slight error become escalated when change is being assessed by differences in the same measure at two points in time. Relatively new techniques have been developed that help both to identify errors and to weigh their magnitude. These techniques require that each measure being employed longitudinally be subjected to a series of statistical treatments. Once the source of measurement error and its extent are known, it becomes possible to control their effects. We have been the beneficiaries of the experience of colleagues who have already mastered the complex demands of these techniques. Even with their help and

guidance, however, we face a very extensive job in honing each of the many measures we shall employ in analyzing change.

At the same time that we have been engaged in methodological tasks, we have pursued our more substantive interests. Part of these interests involve evaluating the effects of a variety of life-events on psychological functioning. The events that appear to produce the most distress are those representing "non-scheduled" role disruptions; that is, events that are not part of the normative unfolding of the life-cycle. The involuntary loss of job, divorce, or the death of a child illustrate events of this type, and they are more likely to result in anxiety and depression than events that are foreseeable well in advance of their occurence.

Our work has also helped to illuminate how life-events and life-changes come to affect people. There is presently a large and growing literature dealing with the consequences of life-changes, and threaded through much of this work is the assumption that change exerts a deleterious impact because it upsets a balance of forces within the organism. Stress, according to this assumption, reflects the psychic effort required to establish and maintain a new inner equilibrium that was destroyed by change. Our own findings indicate that the mechanisms by which life-events adversely affect people are somewhat different. Specifically, there is evidence that the psychological functioning of people is most powerfully influenced when events alter the more durable circumstances of life. Divorce, an event that is generally associated with depression, may be taken as an illustration of this process. What we discover is that divorce results in depression only when there is a concomitant deterioration in economic circumstances, or where increased social isolation accompanies the divorce, or where the newly divorced person becomes solely responsible for the care of young children. Divorce by itself--when it occurs apart from these more persistent conditions--is unrelated to depression. Preliminary examination of a number of events indicates that events by themselves do not lead to emotional distress. Instead, it is the problematic conditions created by the event that generate distress, conditions that may persist long after the occurence of the event itself.

The longitudinal nature of our data has enabled us to test certain propositions concerning patterns of adult development. One such proposition, found especially among theorists holding that the process of aging propels people through well-defined stages, asserts that the middle years of life are a period when various life-crises are especially likely to converge on people. We find very little empirical evidence in support of this proposition. Thus, when different age cohorts are compared, it becomes apparent that middle age is not particularly characterized by hardship and stress. Indeed, young adults are more vulnerable than the middle-aged in this regard. Furthermore, within the group of middle-aged there are wide variations in life-strains and emotional distress. From all indications, the economic and social resources to which people have access are more

instrumental than their ages in explaining psychological functioning. Our findings strongly indicate that age alone can tell us very little about the course of adult development. It is only by also taking into account changes in a host of social and economic characteristics that we can understand the similarities and differences among people as they traverse the life-span.

In the coming year we shall continue to explore the effects of life-events on psychological functioning, seeking to explicate further the mechanisms by which life-changes come to have deleterious consequences. In seeking to clarify this process we shall also be taking into consideration the part played by individual coping efforts and by social support systems.

Publications:

Pearlin, Leonard I. and Joyce S. Johnson: Marital Status, Life-Strains and Depression. American Sociological Review, 42 (October), 1977:704-15.

Pearlin, Leonard I. and Carmi Schooler: The Structure of Coping. Journal of Health and Social Behavior, 19 (March), 1978:2-21.

Pearlin, Leonard I. and Morton A. Lieberman: Social Sources of Emotional Distress. In Simmons, R. (Ed.): Research in Community and Mental Health. Greenwich, Conn., JAI Press. In press.

Rosenberg, Morris and Leonard I. Pearlin: Social Class and Self-Esteem Among Children and Adults. American Journal of Sociology. In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00672-13 LSES

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Social Psychological Correlates of Occupational Position

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. L. Kohn	Chief, Lab. of Socio-environmental Studies	LSES NIMH
OTHER:	C. Schooler	Research Psychologist	LSES NIMH
	J. Miller	Staff Fellow	LSES NIMH
	K. Miller	Staff Fellow	LSES NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH
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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
9.20	3.70	5.50

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The object of this study is to assess the reciprocal effects of occupational conditions and psychological functioning (in particular, values, self-conceptions, social orientation, and intellectual flexibility). Structured interviews were conducted in 1964 with a sample of 3101 men, representative of all men employed in civilian occupations throughout the United States. The study was extended into a longitudinal study in 1974, with the re-interviewing of a randomly-selected one-fourth of the original sample, together with their wives and, where appropriate, one of their children.

PHS-6040
(Rev. 10-76)

Project No. Z01 MH 00672-13 LSES

The principal goal of this research is to assess the reciprocal relationships between occupational conditions and psychological functioning. The research began in 1964 with structured interviews with a sample of 3100 men, representative of all men employed in civilian occupations throughout the United States. These interviews were conducted to our specifications by the National Opinion Research Center of the University of Chicago. In 1974, the National Opinion Research Center conducted follow-up interviews, again to our specifications, with a randomly selected one-fourth of the men who had participated in the original survey. Wherever a man was found to be presently married, a nearly identical interview was conducted with his wife. And wherever a man had one or more children in the age-range 13 through 25, a similar interview was conducted with a previously selected child.

One essential purpose of the follow-up study is to provide more definitive data about causal processes than could be provided by a single cross-sectional survey. With these data, we hope to assess with some precision the magnitudes of the reciprocal effects of occupational conditions and various facets of psychological functioning. The study of wives is designed to ascertain whether occupational conditions affect men and women similarly. These data should also enable us to assess the effects of men's occupational experiences on their wives' psychological functioning and of women's occupational experiences on their husbands' psychological functioning, in each case taking account of the individual's own occupational experiences. The study of the children is designed for exploratory analyses of the effects of parental experiences, values, and practices on their children's psychological development, as well as of the children's own educational and occupational experiences on their own psychological development.

During this past year, our major research efforts have been focused on four objectives: (1) Using the longitudinal data for men, analyzing the reciprocal relationship between occupational structure and intellectual flexibility; (2) Assessing the effects of occupational conditions on the psychological functioning of women; and (3) Assessing the principal determinants of social mobility.

1. Longitudinal analysis of the reciprocal relationship between occupational structure and intellectual flexibility. In last year's annual report, we described the first major longitudinal analysis. This analysis focused on the reciprocal relationship between one pivotal dimension of occupational structure, the substantive complexity of work, and one pivotal dimension of psychological functioning, intellectual flexibility. Our choice of intellectual flexibility to be the aspect of psychological functioning assessed in that analysis was made in part because it offers us the greatest challenge--intellectual flexibility obviously affects recruitment into substantively complex jobs, and there is every reason to expect it to be one of the most resistant to change of all facets of

psychological functioning we have measured. Moreover, intellectual flexibility is so important a part of psychological functioning that we must not unthinkingly assume it to be entirely the product of genetics and early life experience. Rather, we should empirically test the possibility that intellectual flexibility may be responsive to the experiences of adult life.

In that analysis, we developed error-free measurement models for both concepts. Using data derived from those models, we demonstrated that the substantive complexity of work has a decided effect on intellectual flexibility -- approximately one-fourth as great as the effect of the men's 10-year earlier levels of intellectual flexibility. This effect is essentially contemporaneous: The standardized path from 1964 substantive complexity to 1974 intellectual flexibility is a statistically nonsignificant 0.05, while the contemporaneous path from 1974 substantive complexity is a more substantial and statistically significant 0.18.

The reciprocal effect of intellectual flexibility on substantive complexity is even more impressive--surpassing that of the substantive complexity of the 1964 job. This effect is entirely lagged, i.e., it is the men's intellectual flexibility in 1964, not contemporaneously, that significantly affects the substantive complexity of their 1974 jobs.

During this past year, we have continued to focus on intellectual flexibility, but we have enlarged the causal model from one dimension of occupational structure, the substantive complexity of work, to take into account, first, other aspects of occupational self-direction, notably closeness of supervision and routinization, and then occupational structure in general.

Identifying so complex a causal model as that of occupational self-direction and intellectual flexibility is no easy matter. We began by positing that background characteristics that would not be interpreted as job credentials by employers do not directly affect the substantive complexity of the 1974 job, when earlier job history is taken into account. Similarly, we posited that the substantive complexity of pre-1964 jobs should have no direct effect on the men's intellectual flexibility in 1974, but only indirect effects mediated through the 1974 job. Beyond that, we used an incremental strategy, using the paths from each 1964 variable to its 1974 analogue as instruments. We thus initially allowed all contemporaneous paths, but opened the "cross-lagged" paths only as the first-order partial derivatives of the minimization function suggested and the closing-off of nonsignificant contemporaneous paths permitted.

Estimating this model by these incremental procedures, we arrived at a picture similar to, but richer than, that we earlier depicted for the reciprocal effects of substantive complexity and intellectual flexibility. Once again, the effect of substantive complexity on intellectual

flexibility is entirely contemporaneous, and (at 0.17) is approximately one-fourth as large as the effect of the men's earlier levels of intellectual flexibility. The effect of intellectual flexibility on substantive complexity is, again, entirely lagged; in this model, with other occupational conditions affecting substantive complexity, the effect of intellectual flexibility is perhaps a little smaller than the earlier model had estimated it to be (0.36 instead of 0.41); but this is hardly a major change. What is new about the model is that routinization of work and closeness of supervision are shown to have small but statistically significant direct effects on intellectual flexibility, and also indirect effects in that both of them affect substantive complexity. Intellectual flexibility, in turn, has an appreciable lagged effect on closeness of supervision. In sum, the substantive complexity of work is the keystone of an intricately interlocking system: it both affects and is affected by intellectual flexibility more strongly than either of the other components of occupational self-direction. Closeness of supervision and routinization also affect intellectual flexibility, as much through their indirect effects via substantive complexity as through their direct effects.

Next we enlarged the model even further, to consider other important facets of occupational structure. Our surveys include information about more than fifty separable dimensions of occupational structure. Not all of them need be considered here, though, for our earlier analyses indicate that many of these occupational conditions do not have any substantial impact on men's psychological functioning independently of education and of other pertinent occupational conditions. The present analysis focused on fourteen occupational conditions, which together identify a man's place in the organizational structure, his opportunities for occupational self-direction, the principal job pressures to which he is subject, and the principal extrinsic rewards and risks built into his job. Specifically, we indexed three aspects of the man's place in the organizational structure -- ownership, bureaucratization, and hierarchical position; occupational self-direction is of course indexed by substantive complexity, routinization, and closeness of supervision; the job pressures considered here are time-pressure, heaviness, dirtiness, and the number of hours worked in the average week; and the extrinsic risks and rewards are the risk of losing one's job or business, the probability of being held responsible for something outside of one's control, job protections, and job income.

A causal model depicting the reciprocal effects of all these dimensions of occupational structure and intellectual flexibility is even more complex than that for occupational self-direction and intellectual flexibility. Not only is the model several times larger, with many more problems of identifiability, but the relationships among the occupational conditions are themselves complex. In the case of the three components of occupational self-direction, it was reasonable to assume that each could affect the others, both contemporaneously and over time. Thus, in

principle, the substantive complexity of work could be affected by and could affect both routinization and closeness of supervision. But a larger model of occupational structure must recognize that the individual's place in the organizational structure--as defined by ownership, bureaucratization, and hierarchical position--cannot be contemporaneously affected by any other dimension of occupation or by intellectual flexibility. This is definitially true, for a change from ownership to nonownership or the reverse, or from a more bureaucratic to a less bureaucratic firm or organization, or the reverse, or from a higher to a lower position in the supervisory hierarchy, or the reverse, signifies a change in job. It is not even possible for the three aspects of organizational structure to affect each other without it becoming a different job. Lagged effects, of course, are possible, for they can entail moving from one job to another.

We also hypothesized that the contemporaneous effects of ownership, bureaucratization, and hierarchical position on intellectual flexibility are indirect, mediated through more proximate occupational conditions. These are the only a priori restrictions we imposed on the model. We recognized that some other contemporaneous effects are improbable, e.g., it is difficult to see how time-pressure, though obviously correlated with heaviness and dirtiness, could either cause or be caused by them. But it seems wiser to impose the fewest a priori restrictions and let the data determine whether these effects, in fact, occur. We deliberately imposed no a priori restrictions on lagged effects, on the rationale that since people can change jobs, any aspect of a job might influence the individual to change to another job. Thus, to take a trite example, an individual doing heavy or dirty work might be prompted to open a small business. Similarly, intellectual flexibility at an earlier time can affect any aspect of occupation at a later time, even the individual's place in the organizational structure.

In terms of its direct effects on intellectual flexibility, substantive complexity is clearly the most important proximate occupational condition, its contemporaneous effect of 0.13 being decidedly greater than those of income (.05), being held responsible for things outside of one's control (.05), heaviness (-.06), or closeness of supervision (-.03), or than the lagged effects of time pressure (.09) or routinization (-.05). But this is only part of the story. There is an intricate network of interrelationships among occupational conditions, with all of the "structural imperatives of the job" linked to one another and thus, ultimately, to intellectual flexibility. For example, hierarchical position affects substantive complexity, income, closeness of supervision, and job protections, most of which directly affect intellectual flexibility and all of which are linked to still other occupational conditions in complex, reinforcing chains. Thus, substantive complexity in turn affects the probability of being held responsible for things outside of one's control, time pressure, and dirtiness; and closeness of supervision affects

substantive complexity. We need not trace the chains further to demonstrate the complex linkages of occupational conditions. The keystone of the structure is substantive complexity, which directly affects or is affected by almost all of the other occupational conditions. The organizational structure of the job (bureaucratization and hierarchical position, and also supervision and routinization) affect substantive complexity; substantive complexity affects most other actual working conditions.

Since substantive complexity has the greatest direct effect on intellectual flexibility, other occupational conditions gain importance in proportion to the magnitude of their effects on substantive complexity. Thus, hierarchical position, bureaucratization, closeness of supervision, and routinization all indirectly affect intellectual flexibility in proportion to their effects on substantive complexity. There are other indirect routes, but they are of lesser importance. For example, bureaucratization, hierarchical position, and ownership all affect intellectual flexibility through their effect on income. But since income has so limited an effect on intellectual flexibility (a path of .05), any indirect path mediated through income can have only a minor, incremental effect on intellectual flexibility; similarly for indirect effects mediated through closeness of supervision, heaviness, or the probability of being held responsible for things outside of one's control. Judged in terms of its own effect on intellectual flexibility, in terms of its effects on other occupational conditions, or in terms of its role in mediating the effects of other occupational conditions, substantive complexity is clearly the pivotal occupational condition.

Intellectual flexibility's effects on occupational conditions are again entirely lagged. Greater intellectual flexibility is conducive to later working at jobs of greater substantive complexity, with less supervision, and less risk of losing one's job or business, but also with fewer job protections.

Although this model does not permit ownership, hierarchial position, and bureaucratization to have direct, contemporaneous effects on intellectual flexibility, they do have important indirect effects. All three have decided effects on substantive complexity and job income; hierarchical position and bureaucratization also affect job protections and the heaviness of the work; and hierarchical position affects routinization and closeness of supervision. All these proximate occupational conditions, in turn, affect intellectual flexibility -- all but routinization having contemporaneous effects, routinization having lagged effects.

In our continuing work, we plan to assess the reciprocal relationships between occupational structure and several other facets of psychological functioning.

2. Assessing the effects of occupational conditions on the psychological functioning of women. Paid employment is a part of the lives of a large and ever-increasing proportion of women, yet we know little about how women's work affects their psychological functioning and how women in turn affect their work. The purpose of this major segment of our research is to analyze how the principal day-to-day job conditions experienced by women influence and are influenced by their intellectual functioning, self-conception, and social orientation.

Although much is known from our earlier research about the reciprocal relationship between work and personality, all of the evidence is based on men. But in the absence of empirical evidence, we should hesitate to generalize to women conclusions, derived from research on men, about the psychological effects of occupational conditions. Both socialization and social structure may impinge differently on the sexes in powerful and pervasive ways that could make a difference in how men and women affect and are affected by their jobs.

Socialization in childhood and adulthood has been shown to have psychological and behavioral consequences that may directly or indirectly affect the relationship of women to their work. On the most general level, women's socialization has been deemed responsible for such a wide range of real and putative female psychological attributes, values, and behavior that Bernard coined the term "Female World" to describe the specifically female culture that affects women's reactions in all realms of activity, including work. More directly relevant to employment are specific work orientations that have been hypothesized to be the result of differential socialization by sex. For example, women have been described as having a fear of success and emphasizing peer group relationships over work tasks.

Normative expectations, held by men as well as women, that women's occupational interests will be secondary to family roles, may be especially pertinent to the effects of work on psychological functioning. If housework and child care rather than employment are viewed as a woman's primary life responsibilities, the employed woman may experience role conflict and stress that affect her performance in both roles. Even without such role conflict, women who carry dual responsibilities for home and job may become fatigued by the sheer amount of effort needed to fulfill both sets of role requirements. Socialization for the maternal role may influence occupation not only because it may create guilt about working, but also because it may lead to a belief in the necessity of interrupting women's labor force participation during childbearing and pre-school childrearing years. In fact, female labor force participation drops sharply between the ages of 25 and 30, and accelerates again after 30. The interruption of employment and potential careers may influence women's responses to work conditions both before and after the break.

Beyond these attitudinal and behavioral consequences of socialization, there are numerous structural constraints that differentiate women's employment experiences from men's. It is a well established fact that many women work in sex-segregated occupations. Moreover, even for women who work in the same occupations as men, sex discrimination in employment and advancement may influence the relationship of work and personality in women. Women's dollar returns to education and occupational status are lower than men's, even controlling previous work experience and number of hours worked. The possibility of women being at particular stages in their careers at later ages than men, because of child-care interruptions or discrimination, might also produce sex differences in work-personality relationships.

Despite all these real or putative sex differences in socialization and social opportunity, we hypothesized that the psychological effects of occupational experience are generally similar for all adults. Occupational experiences are so important a part of the lives of all employed people that similar experiences should affect the psychological functioning of women in the same way and to as great a degree as they affect men. We therefore hypothesized that employed women's psychological functioning would be meaningfully and substantially affected by their occupational experiences. Moreover, we hypothesized that such relationships between the conditions of work and personality are not solely the result of women being selected into psychologically appropriate jobs or of women molding their jobs to meet their preestablished patterns of psychological functioning. Rather, we expected the causal ordering to be reciprocal, with the conditions and requirements of the job both influencing and being influenced by personality throughout adult life.

Wherever possible, we used maximum-likelihood confirmatory factor analysis to develop error-free indices of principal concepts. We not only developed measurement models of occupational conditions and of intellectual flexibility similar to those developed for the analysis of men, but we extended the range of psychological variables to include the intellectual demandingness of leisure-time activities and several facets of self-conception and social orientation: authoritarian conservatism, criteria of morality, receptivity to change, fatalism, self-confidence, self-deprecation, and anxiety.

Although most of the job conditions in our analysis have substantial zero-order correlations with one or another aspect of psychological functioning, work conditions are so highly interrelated that our first analytic task was to establish which occupational conditions are independently related to each psychological variable, statistically controlling other dimensions of work experience. Multiple regression analyses clearly indicate that the "structural imperatives" of the job have a substantial and meaningful relationship with all facets of women's psychological functioning that we have examined. Moreover, all four types

of structural imperative -- occupational self-direction, organizational locus, job pressures, and job uncertainties -- are independently related to psychological functioning. The multiple-partial correlations for each type of job condition are generally impressive. Particularly impressive are the multiple correlations for the entire set of job conditions.

We also needed to distinguish the impact of occupational conditions on intellectual and personality development from the processes of selective social recruitment and retention in particular occupations. The most obvious prerequisite for employment is education, which in most cases precedes the job and often determines eligibility in particular occupations. Other background characteristics are also involved in occupational selection, to the extent that occupations recruit their members from different segments of society -- drawing more heavily from urban or from rural populations, from particular races, religions, nationalities, or parental backgrounds, or from older or younger members of the labor force. To test whether the relationships between occupational conditions and psychological functioning can be attributed to social characteristics of the workers, we statistically controlled education, race, age, national background, region of origin, urban origin, religious background, parental education, and father's occupational status. When we did this, we found that work experiences are still highly related to intellectual functioning, social orientation, and self-conception. The relationships between occupational conditions and psychological functioning cannot be explained as resulting solely from social selectivity on the basis of education or other social characteristics.

The general magnitudes of the relationships between job conditions and psychological functioning are strikingly similar for men and for women. In only two instances can the overall association between occupational conditions and psychological functioning be statistically differentiated by sex. And in these two cases -- intellectual flexibility and self-confidence -- the relationship is stronger, not weaker, for women. There is no evidence that employed women are in any sense psychologically disassociated from their working lives. Women's intellectual functioning, social orientations, and self-conception are at least as strongly related to their work experiences as are men's.

This analysis indicates that the magnitudes of the relationships between occupational conditions and psychological functioning are considerably greater than previous analyses had shown. The reason is clear: most previous analyses had been based on methods that did not take account of measurement error. Since we find that the magnitudes of relationship are similar for men, when the same methods of index-construction are used, it is clear that we are not dealing with a difference between sexes, but with improved methodology. In this instance, improved methodology yields a substantively important conclusion: the

relationship between occupational conditions and psychological functioning, for women and for men as well, is not only "real", it is very substantial.

Thus, the relationships between occupational conditions and psychological functioning are meaningful and they are robust, in the sense that, in the main, they continue to be statistically significant even when subjected to exceedingly stringent statistical controls. There is the distinct possibility, though, that these relationships, even though statistically significant for the sample taken as a whole, may in reality be conditional on the women's life situations or preferences. This might be the case, for example, if women who are emotionally committed to their jobs were strongly affected by the substantive complexity of their work and women who are less committed to their work were less affected by this job condition. If this were the case, we should draw a very different interpretation of the nature of the relationship between job conditions and psychological functioning than if job conditions affected the more and the less occupationally committed similarly.

We therefore repeated all the multiple regression analyses, systematically testing to determine whether the relationship between any given occupational condition and any aspect of intellectual functioning, self-conception, or social orientation would be much reduced if we statistically controlled a term representing the linear interaction of that occupational condition and occupational commitment. By this method, for example, we can determine whether the relationship between substantive complexity and intellectual flexibility (all other occupational conditions and pertinent background variables statistically controlled) is conditional on whether or not the women are committed to their occupations. We similarly tested each of the occupational conditions vis-a-vis each of the psychological variables. From this systematic test, we learn unequivocally that none of the relationships shown to be statistically significant is conditional on how occupationally committed the women are. These job conditions relate to women's psychological functioning whether or not they are committed to their occupations.

Still, occupational commitment is but one of a multitude of variables that may differentiate women who are more affected by occupational conditions from women who are less affected by those conditions. Five other variables seemed to us to be particularly likely to prove pertinent--the presence of young children in the home, full-time versus part-time work, a preference for being employed versus being a housewife, financial necessity, and the woman's perceived responsibility for contributing to family income. We tested all these possibilities, using the same systematic procedures as we used for testing occupational commitment. Only a scattered few of the relationships -- fewer than would be expected by chance -- are shown to be conditional. The significant interactions are so few and so random that we thought it unprofitable to test other possible conditional effects. We conclude that there is no

evidence that the relationships we have depicted between occupational conditions and psychological functioning are conditional. Rather, these occupational conditions affect women similarly, whatever their situations or their preferences.

Finally, we have been able to show, in a series of causal models, that some key occupational conditions have an actual causal effect on intellectual flexibility, even when exceedingly rigorous statistical controls are imposed, and consistently, in models making a number of different assumptions. Although we have to make further assumptions to identify models of the relationships between occupational conditions and other aspects of psychological functioning, these, too, buttress the interpretation that occupational conditions are not only correlated with, but actually affect, psychological functioning.

These findings should dispel any belief that large segments of the female workforce are somehow immune to occupational circumstances -- that, for example, mothers of young children, or women who do not think of themselves as having careers, or women who are "only working to supplement the family income" are insensitive to the deleterious effects of routinized work or are unresponsive to the mind-stretching experience of doing substantively complex work. Our data strongly suggest that the major dimensions of occupational experience are related to the same aspects of psychological functioning for all types of women, whatever their motives for working or the other circumstances of their lives.

Our findings should also dispel the notion that the relationships between occupational conditions and psychological functioning are not as strong for women as for men. We have shown that these magnitudes are just as great for women as they are for men. Furthermore, with interesting but minor exceptions, the same occupational conditions have similar effects of roughly similar magnitude for women as for men. In particular, both women and men thrive on the opportunity to be self-directed in their work. We have unequivocal evidence that substantively complex, nonroutinized work results in greater intellectual flexibility for both sexes. Our analysis also suggests that these and other structural imperatives of the job are conducive to open-mindedness, personally responsible moral standards, and receptivity to change.

Taken in toto, our findings support our hypothesis that job conditions are substantially related to psychological functioning as much for women as for men. No matter what the sex of the worker, job conditions that elicit effort and flexibility are related to favorable evaluations of self and an open and flexible orientation to others. These findings suggest that the current structure of adult environments impinges on the individual regardless of previous socialization or other life events. Despite sex-role definitions that have traditionally emphasized maternal and marital responsibilities, work histories that are likely to be interrupted,

and part-time work commitments, employed women's intellectual and personality development is contingent on current occupational experiences. The magnitude of the relationship between job conditions and psychological functioning is as strong for women as for men. Women are not insulated by all their other roles from the effects of occupational conditions. Similar life experiences generally have similar effects on both sexes.

3. Assessing the principal determinants of social mobility. The data of the occupations study are also useful for the study of social mobility processes. These data permit the extension of earlier models of the social-structural determinants of status attainment, not only by broadening the range of variables included in the model, but also in their temporal scope. Thus, our model includes variables, such as the substantive complexity of various relevant jobs, that have not previously been examined in relation to status attainment. In addition, it is extended temporally backwards with the inclusion of measures of both grandfathers' occupational statuses. Furthermore, since we have information about the respondent's first job, his 1964, and his 1974 jobs, we can extend the model temporally forward and examine the determinants not only of the status of the individual's first job, but also of subsequent jobs in his career.

We found that grandparents' occupational status levels have significant and moderately strong effects on their children's education, with paternal grandfather's occupational status also having significant direct effects on both the status and substantive complexity of his son's occupation. Of course, this direct effect of grandfather's occupational status on the status and substantive complexity of his son's (the respondent's father's) job is noticeably weaker than the effect on the respondent's father's job of his own education. On the other hand, these effects of respondent's father's education on his own occupational status seem smaller than the parallel effects of the respondent's own education on his occupational status. All in all, the pattern of significant causal relationships among the background variables contains no surprises, but does provide evidence of a moderate degree of status inheritance from the respondent's grandparents to his parents.

Determinants of educational attainment: As has been shown innumerable times, father's occupational status affects education; the findings of the present study, however, raise the possibility that at least part of this previously reported effect of father's occupational status on education may really result from the substantive complexity of the father's job. When the substantive complexity of the father's job is added to the multiple-regression equation predicting education, although the increase in the explained variance is trivial, the standardized regression coefficient of father's occupational status is reduced from .33 to .23, while that of the substantive complexity of the father's work is .13. Thus, educational

attainment is affected not only by the status but also by the substantive complexity of father's occupation.

The present study confirmed earlier findings that family structure affects educational attainment. Coming from a small, or intact, family increases educational attainment, the standardized regression coefficient of family size being the largest of all the determinants of education. As for the relative strength of the effects of mother's and father's education, even for our all-male sample, mother's education has a greater direct effect than does father's. The zero-order correlations of both parents' educational levels with their son's is essentially the same (fathers=.34, mothers=.33). However, when the other variables are added to the model, the effect of father's education is substantially reduced, becoming insignificant when father's occupational status, family size and mother's education are included.

One should, of course, bear in mind that because many of the mothers did not work at a paying job, we did not include either the status or substantive complexity of mother's job in our model. However, when we limited the analysis to respondents whose mothers worked, to examine a model that uses the background variables, including both parents' occupational and educational status, to predict educational attainment, we found that mother's education has a much greater effect than father's education. Furthermore, although father's occupational status has a greater effect on respondent's occupational attainment than does mother's occupational status, the sum of the independent effects of educational and occupational status seems about the same for both parents.

Of the four background variables related to childhood environmental complexity, only urbanicity has a significant positive effect on educational attainment. Surprisingly, when all the other variables are included in the model, age has no significant effect on education. Also worth noting is that in this model neither grandfather's occupational status has any significant direct effect on educational attainment.

Determinants of occupational status: The determinants of the status of the first full-time job the respondent held for six months or longer are the same whether the job is considered in terms of its substantive complexity or its status. Education has the strongest effects on both status and substantive complexity. Religion, region where he grew up, and father's occupational status also affect both job characteristics in similar ways. As might be expected, low father's occupational status and fundamentalist religion result in both low job status and substantively simple work. On the other hand, coming from a region far from the South results in lower occupational status and less substantively complex work. These effects of region on both occupational status and substantive complexity are not those that would have been predicted on the basis of earlier work on the relationship between region and psychological

functioning. However, since the effect of region on occupation takes this form only when education and the other background variables are controlled, the present findings are congruent with those of Blau and Duncan, who note that "...the Southern white benefits from a labor market that is adapted to the educational preparation of the labor force that serves it, allowing him to obtain jobs from which he might well be barred in the North as a result of his educational shortcomings." Since our respondents are likely to have first been employed in the region where they grew up, it is not surprising that when education and the other social background variables are controlled, Southern origins result in relatively high status and substantively complex jobs.

When we examined the determinants of the status and substantive complexity of the 1964 job, we found that education, father's education, and respondent's age have similar effects on both characteristics, education having very strong effects on both. The effects of age are opposite to what might be expected if age were considered only in terms of its psychological consequences. Although the younger members of our sample tend to show greater intellectual flexibility and to have more flexible and self-directed values, they hold lower status and relatively simple jobs. This finding probably results from the younger members of our sample not only being in the earlier stages of their careers, but also tending to be disproportionately concentrated in jobs that do not involve lengthy academic training. The members of their cohort who will eventually be employed in generally substantively complex and high-status occupations are not included in our sample, which was limited to men who were employed.

The substantive complexity of the 1964 job is also affected by two other background variables: urban upbringing and maternal grandfather's occupational status, both of whose effects are in the expected direction. Having an urban upbringing and a relatively high-status maternal grandfather both result in the respondent being employed in a relatively complex job. This direct effect of maternal grandfather's status on the substantive complexity of the 1964 job marks the only time that grandparental status has a significant direct effect on the respondent's educational or occupational attainment. This effect of maternal grandfather's status on the substantive complexity of the 1964 job remains significant even when mother's education is allowed into the model. The existence of such an effect provides tentative evidence that work-relevant attitudes have been transmitted from the maternal grandfather through the mother to the grandson.

Finally, the status and substantive complexity of the first job do not have particularly powerful effects on either the status or the substantive complexity of the 1964 job. The effect of early occupational status on 1964 status is half the effect of education, and the effect of early substantive complexity on 1964 substantive complexity is less than a third that of education. Furthermore, there are no significant

cross-effects; early substantive complexity does not affect 1964 occupational status and early occupational status does not affect 1964 substantive complexity.

Probably because the 1974 job is ten years further along in the respondent's career, none of the background variables continue to have any effect on its status. Instead, all of the significant determinants of this job are the results of the respondent's own previous attainment. Furthermore, the pattern of the effects of earlier attainment on occupational status is different in 1974 from what is was in in 1964. Thus, although the stability of job status from 1964 to 1974 is somewhat greater than that from the first job to the 1964 job, the direct effect of education on the status of the 1974 job is less than half of its direct effect on the 1964 job. Even more striking, although there was no significant path from the substantive complexity of the first job to the status of the 1964 job, the path from the substantive complexity of the 1964 job to the status of the 1974 job is substantial (.36). Even the substantive complexity of the first job has a noticeable direct effect on the status of the 1974 job (.12). Furthermore, this significant effect of the substantive complexity of the first job on the status of the 1974 job takes place in the absence of a significant direct path from the status of the first job to that of the 1974 job. Although there is no significant path from the substantive complexity of the first job to the status of the 1964 job, the path from 1964 substantive complexity to 1974 status is greater than the stability of occupational status from 1964 to 1974. Thus, it would appear that the further an individual progresses in his career, the more important is the substantive complexity than the status of earlier jobs in determining his current occupational status.

Publications:

Kohn, L. and Schooler C.: The Reciprocal Effects of the Substantive Complexity of Work and Intellectual Flexibility: A Longitudinal Assessment. Preprinted in the Harvard-Yale Preprints in Mathematical Sociology Series, No. 9, November, 1977. In press, American Journal of Sociology, 84: No. 1, 1978.

Kohn, M. L. Reassessment, 1977. Pp. xxv-lx of Melvin L. Kohn, Class and Conformity: A Study in Values. Second edition. Chicago, University of Chicago Press, 1977.

Kohn, M. L.: The Effects of Social Class on Parental Values and Practices. In David Reiss and Howard Hoffman (Eds.): The American Family: Dying or Developing. Plenum Press (in press).

Kohn, M. L.: The Substantive Complexity of Work -- and its Relationship to Adult Personality. In Neil J. Smelser and Erik H. Erikson (Eds.): Themes of Love and Work in Adulthood. American Academy of

Arts and Sciences (in press).

Schooler, C. and Smith, K.: "... and a Japanese Wife." Social Structural Antecedents of Women's Role Values in Japan. Sex Roles, 4: 23-41, 1978.

Smith, K. and Schooler, C.: Women as Mothers in Japan: The Effects of Social Structure and Culture on Values and Behavior. Journal of Marriage and the Family, 40: 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00674-08 LSES

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)
Social, Psychological, and Biological Interactions in Schizophrenics and Normals

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	C. Schooler	Research Psychologist	LSES	NIMH
Other:	T. Zahn	Research Psychologist	LP	NIMH
	M. S. Buchsbaum	Research Psychiatrist	APB	NIMH

COOPERATING UNITS (if any)
Laboratory of Psychology, NIMH
Adult Psychiatry Branch, NIMH

LAB/BRANCH
Laboratory of Socio-environmental Studies

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.20	.10	.10

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☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This year has seen the extension of this line of investigation through the use of confirmatory factor analysis to explore the nature of psychophysiological arousal during task performance and the relationship between such activity and amine oxidase activity.

PHS-6040
(Rev. 10-76)

For the past several years, this study has investigated the relationship between blood chemistry and individual functioning.

Publications:

Schooler, C., Zahn, T., Murphy, D. L., and Buchsbaum, M. S.: Psychological correlates of monoamine oxidase activity in normals. Journal of Nervous and Mental Disease 166: 177-186, 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00677-16 LSES

PERIOD COVERED

October 1, 1977 through September 30, 1978

TITLE OF PROJECT (80 characters or less)

HEALTH ORIENTATIONS OF PARENTS AND CHILDREN

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Principal Investigator: John D. Campbell, Ph. D., LSES/NIMH
Research Psychologist

Other: Caren M. Carney, LSES/NIMH
Psychologist

COOPERATING UNITS (if any)

none

LAB/BRANCH

Laboratory of Socio-environmental Studies

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NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

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2	1	1

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☒ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The goals of this research on health orientations of parents and children are first, to describe variations in attitudes, values, beliefs, perceptions, and behavior concerning phenomena of illness and health; and second, to examine how selected personal and situational factors relate to these orientations. Individual perspectives on health, illness and illness-related stress, and the social role of the sick person are to a considerable part learned, and much of this social learning takes place in the interpersonal relationships of family life. Systematic research can shed light on this aspect of socialization, the process by which health orientations are acquired and modified.

PHS-6040
(Rev. 10-76)

Project no. Z01 MH 00677-16 LSES

This research examines children's and mothers' perspectives on illness and health. The principal goals are two: (a) to describe systematically the patterns of variation in family members' attitudes, values, beliefs, perceptions and behavior relevant to illness and health; (b) to examine the links between health orientations and selected personal and situational factors. To accomplish these objectives information was obtained from 264 hospitalized children and their mothers. Interviews with children and mothers, and questionnaires completed by mothers provided data on (a) aspects of their definitions of illness, (b) reports of their own and other family members' feelings and actions when ill, (c) their attitudes and beliefs about illness prevention and treatment, (d) their values in selected areas not directly relevant to health, and (e) their views about appropriate standards of behavior (i.e., what should or should not be done in circumstances pertaining to illness and health).

One of the primary research foci of this study has been the process by which children's health orientations are formed. In previous reports parents have been assigned a critical part in this process. A significant theme emerging in these prior analyses is that parents' general psychosocial orientations (e.g., general values that the parent holds for the child) contribute to the shaping of the sick-role functioning of children. An equally pertinent research issue not previously considered concerns the extent to which aspects of the mother's general orientations are linked to her own functioning in the specific circumstances occasioned by a child's illness. Further development and refinement of indices during the past year has permitted analyses yielding information on this issue.

Social roles, by their very nature, involve reciprocal relationships. Pervasive individual differences in perspectives on these relationships have the potential to contribute to variations in role enactment in situational contexts such as those surrounding illness. Mothers in our study have been found to differ from one another along a dimension that may be referred to as developmental preference, a dimension that appears to capture a portion of the elusive quality of the bond between parent and child: Some mothers think that the time when a child is very young is likely to provide the greatest satisfaction in the parent-child relationship, that maternal satisfactions probably diminish as the child grows up; these mothers would also, if it were possible, "stretch out" the developmental years. In contrast to those with this child-oriented developmental preference, other mothers see the time when a child nears adulthood as likely to offer the greatest potential for satisfaction. Among the mothers in this study, these variations in maternal perspectives, with their implications for the nature of parent-child relationships, showed basically no link with age and sex of child, nor were they a function of the child's health history or current health status. Yet this facet of a mother's general orientations did contribute to her approach to the sick child.

Mothers with a strong child-oriented developmental preference saw themselves as generally very concerned about their child's health. They

placed considerable emphasis on health precautions and were inclined to rely on home medications for the child. (Correspondingly, their children reported that their mothers worried about the child's health and also promptly resorted to use of home medications during the period of the child's illness.) But the readiness with which a mother would seek out a doctor for diagnosis and treatment of the child was not linked to the mother's developmental preference. This suggests that the direct effects of developmental preference on mothers' health-relevant actions pertaining to their children may to some extent be limited to the context of the home, to circumstances in which the mother is clearly functioning as the primary caretaker for the sick child, circumstances in which mother and child are directly involved in role relationships.

The type of involvement evident in a mother who has a strong child-oriented developmental preference may be pervasive and diffuse; in contrast, mothers with a preference for parent-child relationships that more nearly approximate those obtaining between adults may respond to their children in more functionally specific ways. Thus for these latter mothers there should be a consistency in their health-relevant actions that is absent from the actions of the former. Data from the present study support this: The extent to which a mother's concern or anxiety about her child's health is a realistic reflection of the child's health history was greater for mothers emphasizing the satisfaction to be obtained from the more mature child than it was for mothers with a preference for the parent-child relationships of the early childhood years. The link between maternal concern for the child's health and a relaxation of maternal standards for the child's accepting the sick role was also greater for the former group of mothers than for the latter. And the extent to which maternal concern about the child's health was associated with the likelihood of contacting a physician for the child followed this same pattern of contingent relationships. Even children's perceptions of maternal concern or anxiety about the child's health was contingent on the mother's developmental preference: Those who were accurately aware of the degree of their mother's concern for the child's health were predominantly the children whose mothers were favorably disposed toward the child's assuming the adult role. This may have implications for the process by which the child acquires health orientations, because the clarity with which a child can interpret structured experiences with adults can influence social learning.

This research on health orientations provides systematic information on an important area of individual functioning that is currently only inadequately covered. The potential value of the research is not, however, restricted solely to matters of health and reactions to the stressful circumstances surrounding illness. Illness is a universally encountered phenomenon, and there are recognized roles attendant on it. The typically discontinuous, clearly delimited nature of the sick role provides a valuable context in which to consider role learning, role relationships, and other aspects of the acquisition and change of general social orientations.

Publications:

Campbell, J. D. The child in the sick role: Contributions of age, sex, parental status, and parental values. Journal of Health and Social Behavior. 19(1): 35-51, 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER ZO1 MH 00678-01 LSES

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)
The Effects of Adult Life Conditions on Sex Role Ideologies of Factory Workers

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Karen A. Miller Staff Fellow LSES NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Socio-environmental Studies

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.5	.25	.25

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☐ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The analysis estimates a causal model of the effects of adult life conditions, particularly work conditions, on personal sex role ideologies of male factory workers in industrializing countries. The major method of analysis used is maximum likelihood confirmatory factor analysis, which separates error from common variance of concept indicators. Results from analysis in one country, Bangladesh, indicate that work conditions are related to sex role ideologies more strongly than are other adult life conditions.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives

The purpose of this study is to examine how the sex role attitudes of men in developing countries are affected by their contact with industrialization. This is a particular instance of how personal change is associated with social change. The study focuses on some of the day-to-day work conditions of men in factories, and assesses the effects of different aspects of this industrial work experience, independent of other potential explanatory factors, on personal equal rights ideologies. Thus, the study is in a sense complementary to the much broader study of occupational effects on the psychological functioning of U.S. workers, ongoing in the Laboratory.

The analysis estimates a causal model of the effects of adult life conditions on personal sex role ideologies. The major focus is to determine the independent effects of three work conditions -- skill level of the job, machine contact on the job, and benefits of the particular factory environment -- versus other salient life conditions such as educational level, income, consumer goods possessed, and mass media exposure. Also entered in the model are intellectual functioning variables and background variables, either set of which might also have independent associations with sex role ideologies.

Methods Employed

The data used in the analysis reported here consist of interview responses from 654 male factory workers in seven towns and cities in Bangladesh. These data were obtained in 1964/65 by the Project on Social and Cultural Aspects of Development, directed by Professor Alex Inkeles, now at Stanford University. Also available for a potential extension of the present analysis are comparable data from five other developing nations, and data from small subsamples reinterviewed in 1970 in two countries -- Bangladesh and Israel.

For most of the principal concepts in the model, the existence of multiple indicators of the concept makes it possible to use maximum-likelihood confirmatory factor analysis to develop measurement models. This method separates the common variance of indicators from the portion of their variances not shared with the other indicators. The resulting "true scores" allow more accurate estimates of relationships among variables in the model than is possible with methods that do not separate out error.

The dependent variable, sex role ideology, is measured by five indicators, measuring attitudes towards women working, equal pay for equal work, women holding high political office, integration of the sexes in schools and workplaces, and women's rights in general.

Two of the three occupational variables are measurement models. The index of factory environment is constructed from questions about the factory's offering free medical care, housing facilities, bonuses, a recreation program, a library, and other benefits such as paid vacations and on-the-job training, and an independent rating of the factory's overall technical and personnel practices. Skill level is indicated by interviewer-rated, supervisor-rated, and respondent's self-rated skill level. The third occupational variable, machine contact, is a single-item indicator.

Two of the other adult life conditions have multiple indicators. Living standards is indicated by whether or not the respondent possesses each of a list of consumer goods: a watch, radio, bicycle, umbrella, couch, and flashlight. Mass media exposure is indicated by frequency of newspaper reading and radio listening. One of the intellectual functioning variables also consists of multiple indicators -- ratings of answers to six questions about world leaders and places in the form of "Who is --?" and "Where is --?"

Within the measurement models, correlations between the errors of the indicators were estimated when the first order partial derivatives of the maximum-likelihood function indicated that doing so would improve the fit of model to data. All measurement models of more than three indicators were tested using the Chi Square statistic to determine the goodness of fit of model to data. The highest ratio of Chi Square to degrees of freedom was 1.3. Thus, all testable models have excellent fit.

The single item indicators in the model are age, father's education, father's occupation, how many miles from nearest city was place of birth, education, score on oral verbal ability test, score on literacy test, and income.

To estimate the model, the matrix of correlations of true scores and single item indicators was used to do multiple regression analyses. From these analyses, standardized regression coefficients were obtained, which were entered as path coefficients in the model.

Briefly, we model the variables as follows: background variables (where and when born, father's education and father's occupation) are allowed to affect adult life conditions and intellectual functioning, which in turn are allowed to affect sex role ideology. Within the causal ordering imposed by the model, all possible effects are estimated.

Major Findings

From our analysis so far, we have obtained a set of statistically independent relationships among our variables, variables that are free of measurement error where possible. We have modelled

these relationships in a causal structure that must remain hypothetical unless and until we introduce longitudinal data. Although we have not yet done this empirical test, the model is reasonable on theoretical grounds.

The most striking result is that, of all the adult life conditions in the model, only factory environment and skill level of the job have statistically significant direct effects on sex role ideology. Mass media exposure, living standard, and educational level do not have statistically significant direct effects, nor does machine contact on the job. The direct effects of background and intellectual functioning variables are also not statistically significant, with the one exception of father's occupation. In that case, the more urban the father's occupation, the less favorable toward women's rights is the son. The nature of urban occupations a generation ago might explain this result.

Several chains of indirect effects also lead through factory environment and skill level to sex role ideology. Father's education affects son's education, which in turn leads to favorable factory environment (presumably through voluntary or involuntary selection of respondents into factories). Age also positively affects factory environment. Thus father's education, own education and age all work indirectly through factory environment to affect sex role ideology. Variables operating indirectly through skill level are age and father's occupation, the latter having a positive indirect effect while its direct effect is negative.

Significance of Findings and Proposed Course

The tentative conclusion we draw at this point is that two occupational conditions -- skill level of the job and factory environment -- seem to be aspects of the experience of industrialization that are related to the belief that women should have equal rights. That such a relationship between occupational experience and ideology is found at a particular time within a particular sample of Pakistani factory workers is interesting in itself. However, the extension of the analysis to include other cultures and a second interview for some of the same respondents will provide a more rigorous test of our model. An attempt to extend the analysis in these directions will be the next step in the study.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00839-01 LBEB

PERIOD COVERED
October 1, 1977 through September 30, 1978

TITLE OF PROJECT (80 characters or less)
CNS Structures in Mammal-Like Reptiles. I. Forebrain and Epiphyseal Organization

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J.J. Roth	Staff Fellow	LBEB NIMH
Other:	E. Carol Roth	Guest Worker	LBEB NIMH

COOPERATING UNITS (if any)
Dr. Nicholas Hotton, III, Dept. of Paleobiology, National Museum of Natural History, Smithsonian Institution, Washington, D.C.

LAB/BRANCH
Laboratory of Brain Evolution and Behavior

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Poolesville, Maryland 20837

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.2	.4	.8

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The brain space (endocranium) often represents the shape of the brain contained within the neurocranial space. Unfortunately, the brain of mammal-like reptiles (ca 220 mya) is very poorly known (3 genera) because of the lack of prepared material. The striatal complex and epiphysis of the forebrain are, respectively, believed to play an integral role in species-typical behavior and day-to-day thermoregulatory adjustments, yet the evolutionary development of these structures has not been explored. The purpose of this project is to prepare adequate fossil material in generic lines near the reptilian-mammalian transition, and to evaluate changes in brain morphology. In current preparations, it has been shown that some epiphyseal structures (parietal eye) disappear in advanced reptiles (cynodonts). The absence of parietal eyes is believed to reflect internalization of temperature regulation. The contribution of other structures (turbinals, large olfactory bulbs) remain to be evaluated with the addition of more material.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: A thorough analysis of the evolution of the third eye at the Smithsonian Institution (1976-1977) has indicated that very little information exists in regard to the evolution of the mammalian forebrain from reptilian ancestors. Yet the structures of the mammalian forebrain are usually assumed to reflect neural organization among ancestral mammal-like reptiles. Although mammals are undoubtedly descendants of mammal-like reptiles (therapsids), only certain groups of the latter (cynodont lineage) are sufficiently mammalian in terms of skeletal structure to be considered properly as ancestors of modern mammals. Good endocranial casts of the forebrain of mammal-like reptiles do not exist, and only a few hindbrain casts have been reported in the literature; therefore one of the major interests of the Paleoneurology Wing is to prepare new endocranial casts and examine the conformation of the forebrain in contiguous lineages of mammal-like reptiles ancestral to modern mammals. Because gross brain morphology can be correlated with the evolution of animal adaptations, the evolution of the forebrain during the reptilian-mammalian transition should generally reflect the evolution and acquisition of certain categories of adaptation and behavior.

Methods Employed: Well-preserved cranial vaults of certain groups of mammal-like reptiles (e.g., cynodonts, bauriamorphs) selected for their advanced evolution toward mammals have been designated for diamond saw (.010") sectioning by Nicholas Hotton, III, Research Curator, Department of Paleobiology, Smithsonian Institution. Parasagittal sections are made of each skull and the endocranial matrix is removed from the brain space by use of a pneumatic air hammer and a fine drill equipped with carbide burrs. Once fossil skulls are prepared, liquid latex rubber is sequentially applied to the endocranial space, allowed to harden, removed and heat cured. Some forms of latex are sufficiently flexible so that the entire brain cast can be collapsed and withdrawn through the foramen magnum in skulls too fragile or rare to section with diamond saws. The endocranial cast produced is examined for the presence, size, location, and relationship of major brain structures.

Major Findings: To date, examination of existing brain casts indicates that the cerebrum of some advanced cynodonts was small, long and narrow, and that the olfactory bulbs were large. Olfaction, as in living mammals, must have played an important role in prosematic ("nonverbal") behavior. Pineal bodies and third eyes were also prevalent in early mammal-like reptiles, but the third or parietal eye was lost as these reptiles assumed a mammalian habitus. The genetic loss of the parietal eye, but retention of the pineal body, may have been a cogent evolutionary event in species adaptations to changing climatic trends.

Significance to Biomedical Research and the Program of the Institute: Examination of endocranial brain space casts among mammal-like reptiles (225 mya) should yield significant information leading to a more thorough understanding of the evolution of the mammalian brain. Endocranial casts

reflect the temporal acquisition of mammalian brain structure as advanced therapsid reptiles became more mammalian. For example, olfaction plays an important role in mammalian reproduction and communication. The olfactory bulbs in cynodonts are especially large in comparison to living reptiles, suggesting that mammalian olfactory communication was well developed among mammal-like reptiles. In contrast, the cerebral hemispheres are not large in cynodonts and are still reptilian in gross morphology. Existing endocranial casts indicate that the brains of certain mammal-like reptiles do share common morphological and perhaps functional similarities with the brains of living mammals. Important differences will appear as more fossil skulls are prepared.

Proposed Course: To be continued.

Publications: In progress.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00840-01 LBEB

PERIOD COVERED

October 1, 1977 through September 30, 1978

TITLE OF PROJECT (80 characters or less)

Ontogeny of the Epiphyseal Complex in Lizards

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J.J. Roth	Staff Fellow	LBEB NIMH
Other:	E. Carol Roth	Guest Worker	LBEB NIMH
	S. Snyder	Biological Aid	LBEB NIMH

COOPERATING UNITS (if any)

NONE

LAB/BRANCH

Laboratory of Brain Evolution and Behavior

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Poolesville, Maryland 20837

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.6	.1	.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The sequence of events which occur during development of the pineal gland and migration of the parietal eye to the parietal foramen has not been established in lizards. This project records normal development in known-age embryos of the green lizard Anolis carolinensis and several all female populations of parthenogenetic whiptail lizards (Cnemidophorus). Morphological and histological events will form the basis for a reference atlas which will mark appropriate times during ontogeny when neurosurgical techniques will be most successful. The embryological origin of parietal eyes will be established and, as experimental sets of animals mature, age related changes in the central nervous system will be recorded.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: Several published reports have considered specific anatomical questions concerning the normal development (ontogeny) of the parietal-pineal complex, but none have adequately dealt with serial development of the pineal gland or parietal eye (third eye). Fundamental questions considering the origin of parietal eyes and pineal anlage have received only peripheral attention. Eggs incubated in controlled thermal and photic chambers are being used in the Paleoneurology Wing to assess morphological and histological development of the parietal-pineal (epiphyseal) complex throughout ontogeny. When complete, this survey will demonstrate not only the origin and development of the epiphyseal complex, but also its anatomical association with other forebrain structures.

Methods Employed: Mass incubation and hatching of reptilian eggs has been difficult in the past. Recently, with the advent of more precise manipulation of specific biophysical characteristics, reptilian eggs can be cultured successfully. Developing embryos from the unisexual (parthenogenetic) genus Cnemidophorus and representatives of Anolis and Sceloporus are being examined histologically with routine paraffin procedures. In addition, an ultrastructural study of the complex is planned to examine maturational changes in CNS cellular structure. Micrographs will form a reference atlas which will mark opportune times in development to perform neurosurgical manipulations. In this manner the influence of epiphyseal structures on CNS development can be ascertained. Autoradiographic tracings utilizing H^3-thymidine and proline will demonstrate the projections of the pineal body and the parietal eye as well as the time course of development of these structures. Cobalt chloride and horseradish peroxidase are being used to trace afferent connections of the extracranial third eye in parietal eyed species.

Major Findings: A reference set of histological sections is being prepared that will serve as an atlas for intracleidoic surgery. Data on the final destination of pineal gland afferents is also being ascertained.

Significance to Biomedical Research and the Program of the Institute: The present program will provide a neuroanatomical basis for precisely timing surgical intervention in a series of experiments in this laboratory. The embryological origin and dating of photosensory and secretory epiphyseal systems in lizards will also be helpful in gaining a better understanding of the developmental changes occurring in other vertebrates having secretory pineals (mammals and birds).

Proposed Course: To be continued.

Publications: In progress.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00841-01 LBEB

PERIOD COVERED
October 1, 1977 through September 30, 1978

TITLE OF PROJECT (80 characters or less)
Physiological and Ethological Role of the Epiphyseal Complex in Ectothermic Vertebrates

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J.J. Roth	Staff Fellow	LBEB NIMH
Other:	E. Carol Roth	Guest Worker	LBEB NIMH

COOPERATING UNITS (if any)
Dr. William A. Gern, Dept. of Zoology and Entomology, Colorado State University, Fort Collins, Colorado; Dr. Elliott Jacobson, Dept. of Lab. Animal and Wildlife Medicine, U. of Florida, Gainesville, Florida

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.8	.4	.4

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In ectothermic vertebrates (lizards) temperature plays an intergral role in determining the rate at which responses to internal and external stimuli occur. Hyperthermia after parietal eye or pineal body ablation suggests that, in reptiles, these brain structures secondarily influence physiology and behavior by controlling the selection of appropriate body temperatures. This project examines the levels of body temperature which permit the expression of species-typical behavior and physiology in animals lesioned during neonatal development.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: Initial experiments have shown that the epiphyseal complex is intimately involved in the control of temperature selection in certain lizards. Since ectothermic vertebrates are dependent upon external heat sources for the energy to be active, temperature control is a major factor that permeates all facets of reptilian physiology and behavior, i.e., metabolism, reproduction, neuroendocrine interactions and all social interactions involved in reptilian life cycles. One of the hormones (melatonin) produced by the pineal gland has been shown to be an agent that affects temperature selection and hence, reproduction and metabolism. To examine the role of the epiphyseal complex in reptilian life cycles one may utilize surgical manipulations in lizard embryos to interrupt the influence of all or part of the complex before hormonal imprinting occurs. When the embryonically deprogrammed animals achieve maturity, both physiological and ethological alterations of the normal life cycle will be compared with intact animals which should exhibit species-typical behavior. This project can be divided into two components.

1. Physiology - Assessment of temperature control, reproductive capability, and hormonal assays are being conducted.

2. Ethology - Ethograms for maturing experimental individuals will be described and compared with those of normal individuals.

Methods Employed: In adult animals, surgical extirpation of a pineal body is accomplished through a cranial aperture created by a small dental burr (1 mm). In embryonic lizards, the timing of extirpation of the pineal will depend on the results obtained during our ontogenetic project protocol (Project No. 00840-01).

1. Physiology - Temperature regulation and reproduction is being monitored, respectively, by 24-channel Honeywell temperature recorders and appropriate reproductive assays for female lizards (number and size of oocytes; presence of corpora lutea; stage of reproductive cycle; and endocrinological condition). Hormonal assays for melatonin are conducted by Dr. William A. Gern of Colorado State University.

2. Ethology - Ethological data will be gathered and condensed into ethograms for each species and for age groups within each species. Experimental effects of pineal extirpation will be verified by comparing normal ethograms of intact lizards with those of experimental animals. Pineal extirpation will also be verified histologically.

Major Findings: 1. Physiology - Reptilian eggs are being delivered by unisexual and bisexual parietal-eyed and eyeless species of lizards. The period of incubation (45-80 days) depends on the temperature of incubation and the length of the warm phase within each 24-hour thermoperiod. Female Cnemidophorus velox and C. tesselatus apparently have an oviposition cycle

with peaks of oviposition between 2000 and 2400 hours. The peaks of oviposition in an arboreal species, Anolis carolinensis, have a bimodal distribution of 1300 and 2100 hours. Both Cnemidophorus and Anolis are ground nesting lizards, but the former spend the night burrowed in the ground and the latter rest in trees. Endocrinological events (oviducal contraction after oxytocin stimulation) apparently cycle in these two species so that oviposition coincides with suitable nesting sites.

2. Ethology - Ethological comparison of embryonically epiphysectiomized (pineal-less) animals will begin as hatching commences from incubated eggs (ca. 55 days from initiation of incubation).

Virtually all modern vertebrates possess some portion of the parietal-pineal complex except crocodilians, anteaters (edentates), and dugongs (marine mammals). If pineal bodies and the pineal hormone melatonin are integral components of neuroanatomical systems affecting temperature regulation, physiology and reproduction, it is important to ask how some vertebrates can function without the pineal or its hormones. Serum from juvenile alligators was assayed for melatonin. This procedure was carried out (i) because the alligator is a member of the Class Reptilia that does not possess an epiphyseal complex; (ii) because alligators are nocturnal and active when titers of pineal melatonin (a potential activity suppressant) are high in animals with pineals; and (iii) because the Order Crocodilia is the last modern representative of the large subclass Archosauria to which dinosaurs belong. Although pineal bodies are absent in alligators, pineal melatonin without a circadian rhythm was found in the serum (125 picamoles/ per ml serum). Alligators apparently create melatonin from extra-pineal sources (harderian gland) that produce chronic low level amounts in each 24-hour (circadian) cycle.

Significance to Biomedical Research and the Program of the Institute: The findings may suggest that (i) low melatonin levels in nocturnal animals permit greater nighttime activity; (ii) that melatonin is not endemic to pineal bodies; and (iii) since dinosaurs did not have demonstrable pineals, these large reptiles may have also invaded nocturnal habitats. Information about the effects of the epiphyseal complex on temperature regulation, behavior, and reproduction are essential to a better understanding of the ultimate effects of striatal and limbic structures on prosematic ("nonverbal") and other communicative behaviors in reptiles and mammals. In addition, this project will demonstrate the effects of embryonic hormonal imprinting on the programming sequence of adult stereotypic behavior. Evolutionarily, the stability of the pineal hormone melatonin among all vertebrate classes can be contrasted with the genetic loss of the pineal gland proper. Apparently, the gland may be expendable but the pineal hormones are indispensable.

Proposed Course: To be continued.

Publications: In progress.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00842-01 LBEB

PERIOD COVERED

October 1, 1977 through September 30, 1978

TITLE OF PROJECT (80 characters or less)

Comparative Neuroanatomy of the Parietal-Pineal Complex in Lizards

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J.J. Roth	Staff Fellow	LBEB NIMH
Other:	E.Carol Roth	Guest Worker	LBEB NIMH

COOPERATING UNITS (if any)

NONE

LAB/BRANCH

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SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Poolesville, Maryland 20837

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.3	.1	.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Current information on the gross and microscopic anatomy of the parietal-pineal complex among lizards is scarce and inaccurate. This project examines the anatomy of pineal systems and their neural connections with the forebrain in a comparative survey including naturally occurring parietal eyed and eyeless forms. Preliminary analysis indicates that gross anatomical characteristics of the pineal body vary from what has been described in other published reports in the degree of development and amount of physical contact with the parietal eye. Existing errors in the literature derive from inadequate preparation of tissues and sampling techniques which do not reflect the normal histology or morphology of the majority of animals in any given species.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: Both the human pineal gland and other mammalian pineals are often presented in published reports and textbooks as representing the vertebrate pineal in both morphology and physiology. Actually, the mammalian pineal is but one part of a complex of neural structures, including a third eye, which find their fullest development among subavian vertebrates and especially reptiles. Even among reptiles, inadequate sampling and imprecise analysis have led to a recondite anthology of normal pineal morphology. The Paleoneurology Wing is examining both the morphological and histological range of neuroanatomical types of pineal bodies among reptiles. Since secretory pineal systems comparable to those of mammals have evolved among certain lizards, a survey of pineal systems among lizards can be used to compare and derive models for the evolution of the mammalian pineal system.

Methods Employed: The brains of lizards representing naturally occurring parietal eyed and eyeless species are being examined with specific complex. Five individuals from each species will be prepared for referencing the normal amount of variability to be expected in brain morphology from individual to individual. Most sections are prepared from decalcified, paraffin embedded and vacuum infiltrated specimens processed for routine light level histology. Interesting cases will also be examined with electron microscopy to differentiate secretory (pinealocytes) and non-secretory (photosensitive) pineal cells.

Major Findings: Although preliminary, the current findings indicate that the gross morphology of the pineal body in some species differs dramatically from other published (Anolis carolinensis) reports. For example, a forward extension of the pineal body (reported to be absent) exists in A. carolinensis in a significant number of animals. Because the forward pineal extension maximizes exposure of photoreceptive pineal cells by creating a horizontal instead of vertical exposure to transmitted cranial light, the pineal system of A. carolinensis may be neuroanatomically more functional than previously reported.

Although not noted in the literature, ossification of the cranial parietal foramen follows a predictable sequence in animals of increasing snout-vent length (larger and presumably older animals). If the ossification of the parietal foramen in lab-reared animals of known age corresponds to that recorded in increasingly larger animals, a tool will be available for estimating not only age in modern parietal-eyed species of reptiles, but also fossil mammal-like reptiles. This information would make the determination of evolutional sequences more feasible in the lineage of reptiles ancestral to mammals.

Significance to Biomedical Research and the Program of the Institute: This protocol will be important in revealing the evolution of mammalian pineal systems and will be fundamental in establishing a framework for assessing normal pineal morphology and histology among modern reptiles.

Results may be applied not only to modern vertebrates, but may help identify commonalities among ancestral mammal-like reptiles and their descendants, the mammals.

Proposed Course: To be continued.

Publications: In progress.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00851-14 LBEB

PERIOD COVERED
October 1, 1977 through September 30, 1978

TITLE OF PROJECT (80 characters or less)

Brain Mechanisms of Species-Typical, Prosematic Behavior in Squirrel Monkey

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	P.D. MacLean	Chief, LBEB	LBEB NIMH
Other:	T.K. Bussard	Biologist	LBEB NIMH
	R.E. Gelhard	Biologist	LBEB NIMH
	R.M. Sewell	Biologist	LBEB NIMH
	J. Beach	Animal Caretaker	LBEB NIMH

COOPERATING UNITS (if any)

John D. Newman, NICHD, Behavioral Biology Branch

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	.3	1.7

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The present study is part of a long-term comparative investigation testing the hypothesis that the striatal complex plays a basic role in organizing the expression of species-typical, prosematic ("nonverbal") behavior. In mammals, the striatal complex includes the olfactostriatum, corpus striatum (caudate and putamen), globus pallidus, and satellite collections of gray matter. In preceding experiments it was found that lesions of the medial segment of the globus pallidus or interruption of its projections to the ventral thalamus or to the tegmentum affects the expression of the species-typical mirror display of gothic-type squirrel monkeys. Recovery of the display may occur after extensive damage to the thalamic division, but not the tegmental division. The purpose of the present study is to learn which of the tegmental projections is most essential for the expression of the display.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: The present phase of this long-term investigation is concerned with analyzing the role of different projections from the striatal complex (a basic, evolutionary ancient part of the forebrain) in the expression of species-typical, prosematic ("nonverbal") behavior. In preceding experiments, it was shown that lesions of the medial pallidal segment of the striatal complex or certain of its projecting pathways resulted in an enduring elimination or fragmentation of the species-typical mirror display of gothic-type squirrel monkeys. The medial pallidal projections constitute large transhypothalamic pathways anatomically identified as the ansa lenticularis and fasciculus lenticularis. Of these projections, the most important for mediating the display are those to the ventral thalamus (N. ventralis anterior and N. ventralis lateralis) and to the tegmentum. It was shown that recovery of the display may occur after extensive damage to the thalamic division, but not the tegmental division. The cascade of tegmental projections divides into two streams: (1) a dorsomedial outflow that partly terminates in the tegmental area of Forel and (2) a ventrolateral stream that spreads over the substantia nigra and terminates in the N. tegmenti pedunculopontinus in the region of the decussation of the superior cerebellar peduncle. A major purpose of the present work was to learn whether or not it is necessary to interrupt one or both tegmental projections in order to eliminate the mirror display.

Methods Employed: As in preceding years, systematic testing of the mirror display of gothic-type squirrel monkeys is employed for ascertaining behavioral effects of various cerebral lesions (see M-NP-LI-17, 1964). The mirror display is a highly predictable variation of a naturally occurring display used by male squirrel monkeys in a show of aggression, in courtship, and in greeting. Of the five main components of the mirror display, vocalization, thigh-spreading, and thrusting of the erect phallus occur in more than 80 percent of the displays, constituting the so-called trump display. After an animal reaches criterion of 80 percent or more displays in 30 or more trials, electrocoagulations are placed at selected loci of the brain and the monkey is again tested for several months. Pre- and postoperative sound spectrograms of vocalization are also recorded.

Major Findings: An analysis of the role of pallidal transhypothalamic pathways in mirror display has thus far involved systematic observations on more than 50 monkeys with bilateral electrocoagulations of various structures.

In addition to the positive results described in Objectives, an appreciation of the importance of the thalamic and midbrain tegmentum as a funnel for neural elements involved in the mirror display requires a brief enumeration of negative findings: (i) Lesions of large tracts in proximity to the ansal system--namely, the olfactopeduncular tract, mammillothalamic tract, and habenulopeduncular tract--do not interfere with the expression of the display. Except for a diminution of the genital response, lesions of the medial forebrain bundle and inferior thalamic peduncle may have no effect on the incidence of the display. (ii) Destruction of thalamic and

midbrain structures dorsal to the thalamic and midbrain tegmentum, and extending upwards to include the habenula and superior colliculus, may have either no effect or only a transitory effect on the expression of the display.

In regard to the on-going analysis of the role of respective parts of the tegmentum in the display, the following findings are particularly noteworthy:

(1) Far lateral tegmental lesions, including the peripeduncular nuclei, do not interfere with the display.

(2) Partial interruption of the central tegmental tract at the level of the midbrain results in fragmentation of the trump display.

(3) Bilateral partial lesions of N. tegmenti pedunculopontinus have no effect on the display.

(4) Supranigral tegmental lesions may result in a transient or enduring hypothermia, but hypothermia of itself is not sufficient to prevent the display. The evidence suggests that the ventrolateral pallido-tegmental projections are involved in thermoregulation. (Contrary to reported findings in cats and macaques [Ranson and co-workers], complete bilateral destruction of the posterolateral hypothalamus in the squirrel monkey did not result in hypothermia).

(5) Mild bradykinesia resulting from partial interruption of dopaminergic nigrostriatal projections is not sufficient to interfere with the display.

Because of the year-long testing required in some cases, three of this year's animals (L-5, M-5, N-5) are still undergoing testing. The findings on one of these animals should provide further evidence that lesions of the cerebellorubrothalamic system, parts of which are in close proximity to the ventrolateral pallidotegmental outflow, do not permanently interfere with the expression of the display.

In summary, the results of the tegmental analysis thus far indicate that both the dorsomedial and ventrolateral pallidotegmental divisions must be destroyed if the display is to be permanently eliminated.

Significance to Biomedical Research and the Program of the Institute: The results of the present long-term investigation involving behavioral observations on squirrel monkeys have provided evidence that the striatal complex (a fundmental part of the mammalian forebrain that reflects an ancestral relationship to reptiles) plays a basic role in conspecific recognition and the organized expression of ritualistic behavior. The present work dealing with an analysis of the functions of respective tegmental projections from the medial pallidum in display behavior may be expected to contribute to a further understanding of brain mechanisms underlying prosematic ("nonverbal") communication.

Proposed Course: To be continued.

Publications:

Jacobowitz, D.M. and MacLean, P.D.: A brainstem atlas of catecholaminergic neurons and serotonergic perikarya in a pygmy primate (Cebuella pygmaea). Journal of Comparative Neurology, 177(3): 397-416, 1978.

MacLean, P.D.: On the Evolution of Three Mentalities. In Washburn, S.L. and McCown, E.R. (Eds.): Human Evolution. Biosocial Perspectives, Volume 4 of Perspectives on Human Evolution, The Benjamin Cummings Publishing Company, Menlo Park, California, pp. 32-57, 1978.

MacLean, P.D.: The Triune Brain in Conflict. Proceedings of the 11th European Conference on Psychosomatic Research, Heidelberg 1976, Psychotherapy and Psychosomatics, 28: 207-220, 1977.

MacLean, P.D.: Challenges of the Papez Heritage. In Livingston, K.E. and Hornykiewicz, O.H. (Eds.): Limbic Mechanisms. The Continuing Evolution of the Limbic System Concept, Plenum Press, New York, pp. 1-15, 1978.

MacLean, P.D.: An Evolutionary Approach to Brain Research on "Prosematic" (nonverbal) Behavior. In Rosenblatt, J.R. and Komisaruk, B. (Eds.): Reproductive Behavior and Evolution, Plenum Press, New York, pp. 137-164, 1977.

MacLean, P.D.: Effects of lesions of globus pallidus on species-typical display behavior of squirrel monkeys. Brain Research, 149(1): 175-196, 1978.

MacLean, P.D.: A Mind of Three Minds: Educating the Triune Brain. In Chall, J.S. and Mirsky, A.F. (Eds.): Education and the Brain. II. The 77th Yearbook of the National Society of Education, University of Chicago Press, Chicago, Illinois, pp. 308-342, 1978.

MacLean, P.D.: Role of Transhypothalamic Pathways in Social Communication. In Morgane P. and Panksepp, J. (Eds.): Handbook of the Hypothalamus, Marcel Dekker, Inc., New York, (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00856-04 LBEB

PERIOD COVERED
October 1, 1977 through September 30, 1978

TITLE OF PROJECT (80 characters or less)

Role of Paleostriatum in the Species-Typical Display Behavior of Reptiles

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	N.B. Greenberg	Research Associate	LBEB NIMH
	P.D. MacLean	Chief, LBEB	LBEB NIMH
Other:	T.K. Bussard	Biologist	LBEB NIMH

COOPERATING UNITS (if any)

NONE

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	1.1	.4

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The present study on the green Anolis lizard (Anolis carolinensis) was designed as a further test of the hypothesis that the paleostriatum of reptiles, birds, and mammals plays a basic role in species-typical communicative behavior. In preceding experiments, it was shown that lesions of the paleostriatum, but not of the overlying anterior division of the dorsal ventricular ridge (ADVR), resulted in an elimination or a statistically significant deficit in the challenge display of the experimental subject in territorial confrontation with a conspecific male antagonist. In the present concluding experiments, it was found that destruction of the continguous posterior division of the dorsal ventricular ridge (PDVR; "amygdala") did not interfere with the full expression of the challenge display.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: The present study on lizard Anolis carolinensis was designed as a further test of the hypothesis that the striatal complex plays a basic role in organizing the expression of complex species-typical behavior. Developments in neurochemistry and histochemistry have helped to identify the corresponding striatal structures in reptiles, birds, and mammals. In reptiles and birds, the striatal complex lies beneath the dorsal medullary lamina. In lizards, the submedullary structures include the olfactostriatum of Cairny and the paleostriatum of Ariëns Kappers. These paleostriatal structures are to be distinguished from the overlying anterior and posterior divisions of the dorsal ventricular ridge of Johnston, corresponding, respectively, to the neostriatum and archistriatum (amygdaloid complex) of Ariëns Kappers. In last year's report it was explained that lesions of the paleostriatum, but not of the overlying DVR, resulted in an elimination or marked deficit in the performance of the challenge display. The present concluding experiments deal with the question of whether or not a lesion confined to the posterior division of DVR (amygdaloid complex) would interfere with the challenge display.

Methods Employed: The method for testing pre- and postoperative display behavior in mature male Anolis carolinensis was described in a previous project report (Z01 MH 00854-03 LBEB). Since there is an almost complete decussation of the optic nerves in this species, a lesion can be placed in one hemisphere and the lizard's response to a conspecific male antagonist in territorial confrontation can be tested with either eye covered. In addition to not interfering with thermoregulation, the unilateral procedure has the advantage that the intact hemisphere can serve as a "control" for assessing both the behavioral changes and cerebral damage. Planimetry is used for obtaining volumetric measurements of the amount of cerebral damage.

Major Findings: Success was achieved in one animal in placing a lesion confined to the posterior division of the dorsal ventricular ridge. Although the destruction did not exceed 16% of the volume of this structure, the lesion was so placed as to disrupt its major connections. When vision was confined to the eye projecting to the damaged hemisphere, there was no significant alteration in the performance of either the signature or challenge displays.

Significance to Biomedical Research and the Program of the Institute: In the squirrel monkey, complete bilateral destruction of the amygdala is compatible with the regular performance of the mirror display. The present experiments on lizards provide further comparative evidence that destruction of the amygdala is compatible with the regular performance of ritualistic displays. On the basis of the evidence accumulated thus far, there is evidence that in animals as widely separated as reptiles and primates, the paleostriatum is basically involved in organizing the expression of displays used in prosematic ("nonverbal") communication (see Z01 MH 00851-14 LBEB).

Proposed Course: The first phase of this work has been completed and a paper describing the results has been submitted for publication.

Publications:

Greenberg, N.B. and MacLean, P.D. (Eds.): The Behavior and Neurology of Lizards, U.S. Government Printing Office, Washington, D.C., DHEW Publication No. (ADM) 77-491, 1978, 338 pp.

Greenberg, N.B.: Ethological Considerations in the Experimental Study of Lizard Behavior. In Greenberg, N. and MacLean, P.D. (Eds.): The Behavior and Neurology of Lizards, U.S. Government Printing Office, Washington, D.C., DHEW Publication No. (ADM) 77-491, 1978, 338 pp.

MacLean, P.D.: Why Brain Research on Lizards? In Greenberg, N. and MacLean, P.D. (Eds.), The Behavior and Neurology of Lizards, U.S. Government Printing Office, Washington, D.C., DHEW Publication No. (ADM) 77-491, 1978, 338 pp.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00861-04 LBEB

PERIOD COVERED
October 1, 1977 through September 30, 1978

TITLE OF PROJECT (80 characters or less)
The Neuroethology of Reproductive Behavior: I. Role of the Accessory Olfactory System

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M.R. Murphy	Senior Staff Fellow	LBEB NIMH
Other:	T.K. Bussard	Biologist	LBEB NIMH
	S.C. Hamilton	Biologist	LBEB NIMH

COOPERATING UNITS (if any)
NONE

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NIMH, ADAMHA, NIH, Poolesville, Maryland 20837

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.3	.1	.2

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The importance of the accessory olfactory system (vomeronasal system) to the mating behavior of male hamsters is being studied. Animals initially failing to mate following lesions of the vomeronasal nerves recovered after 3 to 6 weeks. Histological examination of the accessory olfactory bulb demonstrated a complete lesion of the vomeronasal nerve in 10 out of 11 subjects.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: The vomeronasal system is a chemosensory system present in most species of terrestrial vertebrates; its specific functions remain unknown. In previous studies, lesions of the vomeronasal nerve were found to have a discrepant effect on the mating behavior of male hamsters, leaving some animals seemingly unaffected and others deprived of the ability to copulate. The purpose of the current project is to determine if any aspect of mating behavior is consistently affected by lesions of the vomeronasal nerve. The behavioral results of this study were reported last year. This present report describes the results of the examination of the lesions.

Methods Employed: To assess the completeness of the section of the vomeronasal nerves, the brains of the 11 lesioned subjects were cut in the frontal plane at a thickness of 50 microns and successive sections were stained alternately for Nissl substance and myelin. In normal material the olfactory glomeruli of the accessory olfactory bulb (projection area of the vomeronasal nerve) are not visable in the Nissl stain, but the small periglomerular cells stain blue and are organized in semicircles around the invisible glomeruli. In the myelin stain the glomeruli stain light brown and collectively have the appearance of a cluster of grapes. Microscopic changes in the accessory olfactory bulb were plotted on drawings of every second section projected at a magnification of X 40.

Major Findings: It was reported last year that, except for slight changes in grooming and intromission patterns, the mating behavior of nine of the lesioned males was normal. Two other males initially failed to copulate, but recovered normal behavior in 3 to 6 weeks. Microscopic examination of the lesions revealed that one of the 9 males which mated retained normal appearing olfactory glomeruli in the accessory olfactory bulbs on both sides of the brain. The behavior of this animal was not significantly different from the behavior of animals with complete lesions of the vomeronasal nerve.

Significance to Biomedical Research and the Program of the Institute: Cumulative evidence from this and other laboratories indicates that the vomeronasal system is important to the mating behavior of hamsters and other mammals. Unique behavioral significance of this chemosensory system, independent from the main olfactory system, has yet to be determined.

Proposed Course: To be continued.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00863-04 LBEB

PERIOD COVERED

October 1, 1977 through September 30, 1978

TITLE OF PROJECT (80 characters or less)

Social Preference Behavior

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M.R. Murphy	Senior Staff Fellow	LBEB NIMH

COOPERATING UNITS (if any)

NONE

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NIMH, ADAMHA, NIH, Poolesville, Maryland

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.3	.2	.1

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The variations in the social preferences and social motivation were studied in relation to changes in the reproductive condition of female Turkish hamsters. When offered a choice between a conspecific male and a male from a closely related species, cycling females displayed high investigation of males and a strong preference for conspecific males on all four days of the estrous cycle. In addition to differential investigation, preference was displayed by vaginal scent marking in front of the preferred male. This form of marking is thought to be a sexual solicitation behavior. Both total interest in males and preference for conspecific males declined after impregnation, and were lowest during the second half of pregnancy and the first half of lactation. Both behaviors began to return during the last half of lactation. Females in a naturally occurring acyclic state also failed to show social preferences.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: This project deals with the fundamental question of what neural and other factors are critical for mammals of a particular species to recognize and associate with their own kind. In previous studies it was determined that estrous female Turkish hamsters (Mesocricetus brandti) display strong preferences for conspecific males over males of a closely related species, Syrian hamsters (M. auratus). The purpose of the current experiment was to determine how the social preferences of female hamsters vary with their reproductive condition.

Methods Employed: Social preferences were assessed by observing the subjects' behavior toward a pair of active male hamsters; one male was a conspecific Turkish hamster and the other male was a Syrian hamster. Males were restricted behind barriers so that females could see, hear, and smell, but not touch them. Of the measured variables the most significant were (i) total interest in males, (ii) total vaginal scent marking, (iii) percent of total interest in males directed toward the conspecific male, and (iv) the percent of vaginal scent marks made in front of or immediately after investigating the conspecific male. The social preferences of different groups of females were studied during the estrous cycle, pregnancy, lactation, and a naturally occurring acyclic condition.

Major Findings: Female Turkish hamsters displayed high interest in males and a strong preference for conspecific males on all four days of the estrous cycle. In addition to differential investigation, preference was displayed also by vaginal scent marking in front of the preferred male. This form of scent marking is believed to be a sexual solicitation behavior. Both the total interest in males and the preference for conspecific males declined after impregnation and were lowest during the second half of pregnancy and the first half of lactation. Vaginal scent marking was almost absent throughout pregnancy and the first half of lactation. Interest in males, frequency of vaginal scent marking, and the preferential display of these behaviors toward conspecific males began to increase during the last half of lactation, presumably in anticipation of resumed cycling. Females in a natural acyclic state displayed decreased scent marking and an absence of social preference but, in contrast to pregnant and lactating females, showed high interest in males.

Significance to Biomedical Research and the Program of the Institute: Species recognition and social preferences are fundamental aspects of animal behavior that have been neglected. Social preferences are critically involved in the evolution of sexual dimorphism, the origin of species, the sexual isolation of species, and the establishment and maintenance of social structure. The findings reported here illustrate non-copulatory behaviors changing in a logically adaptive manner with reproductive condition. These behaviors and the changes they undergo are species-typical characteristics of the hamster. The description and analysis of such behavior forms the base on which studies of the hormonal and neural mechanisms of behavior can build.

Proposed Course: To be continued.

Publications:

Murphy, M.R.: Interspecific sexual preferences of female hamsters. Journal of Comparative and Physiological Psychology, 91: 1337-1346, 1977.

Murphy, M.R.: Estrous Turkish hamsters display lordosis toward conspecific males but attack heterospecific males. Animal Behaviour, 26: 311-312, 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00864-04 LBEB

PERIOD COVERED

October 1, 1977 through September 30, 1978

TITLE OF PROJECT (80 characters or less)

The Role of the Biogenic Amines in Aggressive and Reproductive Behavior

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: M.R. Murphy Senior Staff Fellow LBEB NIMH

COOPERATING UNITS (if any)

NONE

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.05	.05	

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In this project the effects of simultaneous stimulation of the dopamine system and impairment of the olfactory system on the social behavior of male hamsters are being studied. It has been found that apomorphine, a putative activator of dopamine receptors, greatly inhibits copulatory behavior and increases aggressive behavior in sexually active male hamsters. Apomorphine also increases aggression in olfactory bulbectomized males, indicating that the drug acts independently of the normal olfactory sensory control of aggression in hamsters. The only animals failing to show a response to apomorphine were four males in which lesions of the sensory nerve supplying the accessory olfactory bulbs had temporarily eliminated mating behavior. Lithium chloride which, like apomorphine, causes gastrointestinal distress, had no effect on the mating behavior of hamsters.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: The purpose of the present project is to test the effects of simultaneous stimulation of the dopamine system and impairment of the olfactory system on mating and aggression of hamsters. The entire olfactory apparatus was eliminated in some animals, and only the accessory system in others. The behavioral results of this project were reported last year. This year the project was completed with histological examination of the lesions and the addition of a group of animals to control for the aversive effects of apomorphine, the drug used to stimulate the dopamine system. Apomorphine has an emetic effect on animals that have a vomiting reflex and produces gastrointestinal distress in other animals; it is often used as the "poison" in studies on bait shyness in rodents. To control for the possibility that the aggression seen in hamsters following apomorphine was a reaction to the aversive qualities of the drug, 10 additional hamsters were tested for copulatory performance following an injection of lithium chloride, an agent also used in experiments on bait shyness.

Methods Employed: The histological methods are reported in Progress Report Z01 MH 00861-03. Lithium chloride (.2 molar) was injected intraperitoneally at a dosage of 15 ml/kg. This dose is known to be sufficient to induce bait shyness in hamsters.

Major Findings: As described in an earlier report (Z01 MH 00864-03), injections of apomorphine resulted in greatly increased aggression in all hamsters tested except for 4 animals that had stopped mating following a lesion of the vomeronasal nerve. Since that report histological examination has been completed. Normal appearing olfactory glomeruli were found in only one of the 16 animals that had been subjected to vomeronasal section. A few normal appearing olfactory glomeruli were found in the brain of one of the animals in which the olfactory bulbs had been removed. Neither of these animals were significantly different from corresponding subjects with complete lesions. Injections of lithium chloride did not induce aggressive behavior in any hamster.

Significance to Biomedical Research and the Program of the Institute: The main olfactory system, the vomeronasal system, and dopamine are all involved in the control of social behavior in hamsters. Neural areas of possible interaction of these systems include the olfactory tubercle, which receives projections from the mesolimbic dopamine system and the main olfactory bulb, and the bed nucleus of the stria terminalis, which contains dopamine receptors, receives an input from the accessory olfactory bulb, and is known from other studies to be involved in aggressive behavior.

Proposed Course: To be continued.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00869-02 LBEB

PERIOD COVERED
October 1, 1977 through September 30, 1978

TITLE OF PROJECT (80 characters or less)

The Striatal Complex: Question of Role of Dopamine and Opiate Receptors in Species-Typical Behavior of Canids

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	P.D. MacLean	Chief, LBEB	LBEB NIMH
	M.R. Murphy	Senior Staff Fellow	LBEB NIMH
Other:	R.M. Sewell	Biologist	LBEB NIMH
	R.E. Gelhard	Biologist	LBEB NIMH
	G.B. Kerlin	Biological Aid	LBEB NIMH

COOPERATING UNITS (if any)

C. Pert and D. Bowie, Biological Psychiatry Branch, NIMH

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.9	.2	.7

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The present study is concerned with the effects of the chronic administration of blockers of dopamine and opiate receptors on developing dogs. The findings on the pimozide-treated animals were of particular interest, because in addition to the changes in the species-typical behavior and hypersensitive response to apomorphine described in last year's report, these animals were the only ones to show delayed estrus and failure in reproductive behavior. The neurochemical analysis has not yet been completed.

Project Description:

Objectives: In planning for the present laboratory, a main purpose was to test the hypothesis that the striatal complex plays a basic role in organizing the expression of complex, species-typical behavior. The striatal complex constitutes the fundamental part of the basal forebrain in all terrestrial vertebrates. Parts of the striatal complex are rich in dopamine and opiate receptors. The purpose of the present experiments was to learn whether or not the chronic administration of pharmacological blockers of dopamine and opiate receptors would affect the unfolding of species-typical behavior in developing canids. Dogs were used in these experiments because they exhibit not only species-typical, but also breed-typical behavior. The ethological and other behavioral observations were described in last year's report (00869-01). The present report includes data on estrus and breeding that was not available at the time of the previous report, as well as the initial results of the neurochemical analysis.

Methods Employed: Pimozide and naltrexone were chosen as pharmacological blockers, respectively, of dopamine and opiate receptors. Half of one litter of 8 beagle puppies was treated with pimozide beginning at about two weeks of age; the other half served as control subjects. Another litter of 8 puppies was similarly employed for testing the effects of naltrexone. Drug treatment continued for a period of 8 months. Adequate dosage was periodically confirmed by challenges with apomorphine and with methadone. The presence of estrus was detected by obtaining vaginal smears at prescribed times. At the termination of the experiment (about 300 days after withdrawal from drugs) animals were rapidly anesthetized in a chamber for delivering gas and absorbing CO_2. A vibrating macrotome was used to obtain serial half cm slices cut normal to the neuraxis. Samples of fresh brain tissue were then dissected from 15 representative structures. Opiate assays were done by the method of C. B. Pert et al (Nature, 269, 73, 1977) and the dopamine receptor assay by the method of D. R. Burt (Mol. Pharmacol., 12, 800, 1976).

Major Findings: As described in last year's report, the four pimozide-treated animals developed several behavioral aberrations, including the failure to engage in allelomimetic behavior and failure in field tests. When challenged by increasing doses of apomorphine, they proved to be protected against the emetic effects, but they engaged in prolonged stereotypic behavior suggestive of a hypersensitive reaction. One month after withdrawal, they reacted positively to subemetic doses of apomorphine.

Except for some evidence of precocious sexual behavior, the naltrexone-treated dogs were not remarkable.

I. Estrus and breeding: A total of 11 females were followed from August 1976 to the spring of this year. Drug treatment was discontinued about 300 days after birth. There were two pimozide-treated dogs and three controls from one litter; two naltrexone-treated dogs and two controls from another litter; and one methadone-treated and one control from a litter

observed in an ancillary experiment. All except the two pimozide-treated females came into estrus within 50-52 weeks, falling within the standard range for beagles (49.7-52.3 weeks). One pimozide-treated animal came into estrus after 62 weeks and the other after 80 weeks, representing deviations from the mean of statistical significance. All but the pimozide animals became pregnant, whelped, and reared their young. One pimozide dog delivered prematurely 7 stillborn pups, and the other female was sacrificed after coming into heat in 80 weeks.

II. Neurochemical findings. The initial results of the neurochemical analysis showed no significant differences between the control and treated animals. Thus far assays have been done only on the nucleus accumbens, the caudate nucleus, and the amygdala.

Significance to Biomedical Research and the Program of the Institute: In the foregoing study, the results of the chronic administration of pimozide to developing beagles were of particular interest. In addition to the species-typical changes described in last year's report, the animals showed an unexpected reaction to apomorphine in that they were hypersensitive to its stereotypy inducing effect during drug treatment and hypersensitive to its emetic effect one month after withdrawal. Unlike the control and naltrexone-treated animals, the two pimozide-treated females showed significantly delayed estrus, an effect perhaps attributable to the action of the drug on dopamine cells of the arcuate nucleus. The single animal to become pregnant delivered premature stillborn pups. The 9 remaining females in the study all came into estrus at the expected time, whelped successfully, and reared their young.

Proposed Course: Concluded.

Publications: None.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00870-02 LBEB

PERIOD COVERED
October 1, 1977 through September 30, 1978

TITLE OF PROJECT (80 characters or less)
Species-Typical Behavior of Hamsters Deprived From Birth of Neocortex

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M.R. Murphy	Senior Staff Fellow	LBEB NIMH
	P.D. MacLean	Chief, LBEB	LBEB NIMH
Other:	S.C. Hamilton	Biologist	LBEB NIMH
	T.K. Bussard	Biologist	LBEB NIMH
	N. Bohlen	Guest Worker	LBEB NIMH
	L. Lowe	Guest Worker	LBEB NIMH
	A. Kerlin	Biological Aid	LBEB NIMH

COOPERATING UNITS (if any)

NONE

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.7	.6	2.1

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In an investigation of functions of the striatal complex and the limbic system, ethological observations have been made on the development of hamsters deprived since birth of the neocortex. Such preparations grew and developed like normal animals, showing the usual species-typical behavior, including successful mating and breeding. The findings are at variance with views that the neocortex is requisite for the expression of such complex behavior in mammals.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: The purpose of this project is to test the hypothesis that the two older evolutionary formations of the forebrain (the striatal complex and the limbic system) are sufficient, along with the remaining neuraxis, for the expression of most forms of species-typical behavior.

Methods Employed: Hamsters are primarily used for this work because they have a short gestation period and display many natural forms of behavior in a laboratory setting. For comparative purposes, observations are also made on rats. Three procedures are used for eliminating the neocortex: (i) thermal destruction of the developing cortex on the first or second postnatal day; (ii) aspiration of the cortex by microsurgical techniques; (iii) destruction of cortical neuroblasts by prenatal administration of methylazoxymethanol acetate (MAM). Behavioral development is followed and recorded by the use of a checklist derived from an extensive ethogram. Quantitative measures of mating and other behavior are obtained with the use of a computer-assisted event recorder and time-lapse television. The postmortem examination includes craniometry, examination and weighing of glands of internal secretion, and preparation of serial sections of the brain.

Major Findings: The surgical procedures have proved preferable to the use of a chemical in eliminating the neocortex because (i) there is no complicating morbidity of the mother and necessity of cross-fostering pups; (ii) there is no direct deleterious effect on other developing organs; and (iii) littermates may serve as control subjects.

Several male and female hamsters with virtual destruction of all neocortex have been followed for several months. In some, there was also complete destruction of the limbic cingulate cortex and partial damage to the hippocampus. To date, MAM has been administered to 40 pregnant hamsters and four pregnant rats in an attempt to ascertain the optimum dose and time of treatment for eliminating the neocortex. Thus far, with different schedules for administering the drug on fetal days D11 through D15, the frontal cortex failed to show the advanced degeneration seen in the parieto-occipitotemporal neocortex. In rats, the recommended doses for eliminating neocortex resulted in only moderate micrencephaly.

The observations on additional animals prepared this year, including MAM-treated hamsters with moderate micrencephaly, have confirmed and extended our previous findings concerning the retention of species-typical forms of behavior occurring under laboratory conditions (see Z01 MH 00870-01 LBEB). In addition, it has been quantitatively demonstrated by the use of time-lapse TV that hamsters without neocortex show significantly more stereotyped pacing back and forth than do controls. Such animals have also proved uniformly to be more difficult to handle than the controls.

Further information has also been obtained about the capacity of these animals to mate and breed. One female in which both the neocortex and midline limbic cortex were lacking was of particular interest: in test situations she flank-marked significantly more than controls and was also atypical in presenting to a male which she could not touch. Although successfully rearing 6 of 7 pups and appropriately covering them in the nest, she never retrieved them. She defended her nest with unusual ferocity.

Neuroanatomical findings. In animals without neocortex there was an absence of the corpus callosum and pyramids. A foreshortening and great narrowing of the thalamus reflected the virtual disappearance of ventral and lateral nuclei. In cases in which the midline limbic cortex was absent on one or both sides, there was a reduction in the size of the anterior nuclear group. In the striatal complex, Wilson's pencils were preserved and the pallidal outflow (lateral forebrain bundle) was well preserved, including the massive projections to the tegmentum and substantia nigra.

Significance to Biomedical Research and the Program of the Institute: The findings in these and previous experiments (see Z01 MH 00870-01 LBEB) that all forms of species-typical behavior find expression in animals deprived of neocortex since birth, run counter to the established view that "with the amplification of cortex in lower mammals [complex instinctive functions]...seem to have been taken over wholly by the cortex" and that the mammalian striatum "seems to be a relict of a much more diversified organ" (C.J. Herrick, 1926). That one female with additional loss of the midline limbic cortex failed to retrieve her young is of particular interest in view of the possibility that the evolutionary development of this cortex in mammals partly reflects an added dimension in parental care.

Proposed Course: To be continued.

Publications:

Murphy, M.R.: A Table of Hamster Behavior. In Inbred and Genetically Defined Strains of Laboratory Animals, FASEB Handbook, (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00871-02 LBEB

PERIOD COVERED

October 1, 1977 through September 30, 1978

TITLE OF PROJECT (80 characters or less)

Comparative Histochemical Analysis of the Ventral Striatal Complex

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R.C. Switzer, III	Senior Staff Fellow	LBEB NIMH
Other:	S. Berga	COSTEP	LBEB NIMH

COOPERATING UNITS (if any)

NONE

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.4	.2	.2

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The deep lying, large output neurons of the olfactory tubercle have been implicated as being pallidal-like; therefore it seems likely that this region of tubercle could be a rostro-ventral extension of the globus pallidus. Evidence exists that pallidal neurons concentrate iron in the form of proteinaceous ferritin, and therefore may be uniquely identified using immunocytochemical methods. Since normal histochemical techniques designed to demonstrate iron have failed, probably because of the state of the iron within the ferritin molecule, the immunocytochemical method is being pursued to detect the ferritin. This method requires (i) the extraction of ferritin from rat tissue; (ii) injection of ferritin as antigen into rabbits; (iii) extraction of antibodies from rabbit serum; (iv) column chromatography of antibody sera to increase the specificity of the antibody-antigen reaction; and (v) application of sera to forebrain sections for demonstration of ferritin-containing cells. Other histochemical studies showed that: a) The acetylcholinesterase (AChE) stain of a skate brain to be of low intensity in striatum but heavy in brainstem areas; b) Although striatal AChE in opossum appeared typical, distinct patterns were present in frontal cortex and the trigeminal nucleus.

Project Description:

Objectives: Various areas of the brain are distinguished by their intrinsic chemical composition as revealed by appropriate histochemical or immunochemical techniques. The present experiments are designed to:

I. Test the hypothesis that globus pallidus-type neurons lie within the olfactory tubercle. These neurons will be examined with an immunochemical method for their content of ferritin--an iron-containing protein which is in high concentration in main globus pallidus.

II. Document and compare the distribution of acetylcholinesterase in different vertebrates as they become available--brains from a skate (a ray-like member of the same subclass as sharks) and an opossum were examined.

Methods Employed: I. The presence of ferritin is to be determined by using an immunochemical tag, an antiferritin antibody. Insufficient cross reactivity between rat ferritin and commercially available horse antiferritin necessitates extracting our own rat ferritin, purifying it and using it to generate antibodies in rabbits. Following purification, the antibodies from rabbits will be applied to cryostat-sections of the rat brain.

II. Sections obtained from either formalin or paraformaldehyde-glutaraldehyde perfused brains are processed by a modified Koelle method for acetylcholinesterase employing a silver intensification step. Some sections are incubated in the standard way at 37°C for periods of 30-90 minutes, while other sections are incubated at 4°C for approximately 17 hours.

Major Findings: I. In preliminary experiments, fresh as well as fixed brain sections were treated for iron, ferritin, or glutamate dehydrogenase using standard histochemical methods. Each method proved to be insensitive for localizing the respective substance tested. Immunodiffusion tests using antihorse ferritin and rat ferritin confirmed earlier reports of their poor cross reactivity. The ferritin extracted from the liver and spleen of rats, whose drinking water was high in iron-citrate, is being purified with column chromatographic methods before injecting the ferritin antigen into rabbits.

II. Acetylcholinesterase (AChE) in the striatal complex of the skate was weak or absent. Identification of other areas which did exhibit intense staining will be dealt with elsewhere.

The AChE pattern in striatum and ventral striatum of opossum appeared to be the same as the pattern seen in rat. However, two ancillary findings of interest were seen elsewhere in opossum.

In the principal nucleus of the trigeminal complex, a pattern exists which is similar to that seen in the area of cortex (cell bodies) and in the thalamus (succinate dehydrogenase activity), where the facial vibrissae

are represented. A distinct patch of AChE activity in suprarhinal frontal cortex (not seen in rat) suggests the special functional difference of this area, possibly having some relevance to the adjacent suprarhinal olfactory cortex (see Z01 MH 00872-02 LBEB).

Significance to Biomedical Research and the Program of the Institute: I. The data to be obtained from this study will be useful in providing a more complete characterization of the striatal complex. Knowing the detailed localization of ferritin in striatum may provide a means of explaining why areas affected in Huntington's disease are regions normally high in both ferritin and GABA content (regions with high GABA or ferritin alone are less significantly affected). The olfactory tubercle has recently aroused greater interest because of the discovery that it contains a high amount of opiate and estrogen receptors.

II. The weak staining for AChE in the skate brain may indicate another esterase is employed in the skate's cholinergic metabolism, e.g., butyryl-cholinesterase. This could be tested by using the same method as for AChE but with a different substrate (butyrylthiocholine) and a different nonspecific cholinesterase inhibitor.

Proposed Course: To be continued.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00872-02 LBEB

PERIOD COVERED

October 1, 1977 through September 30, 1978

TITLE OF PROJECT (80 characters or less)

Comparative Study of Olfactory Projections to Neopallium

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R.C. Switzer, III	Senior Staff Fellow	LBEB NIMH
Other:	J.M. Hill	Biologist	LBEB NIMH

COOPERATING UNITS (if any)

NONE

LAB/BRANCH

Laboratory of Brain Evolution and Behavior

SECTION

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NIMH, ADAMHA, NIH, Poolesville, Maryland 20837

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.5	.3	.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The olfactory bulb projects directly to the neocortical part of insula cortex in opossum, while the olfactory projections to a similar zone in rat are mediated by piriform cortex. The thalamic relationships of the olfactory zone of neocortex in opossum were sought by injecting horseradish peroxidase (HRP) (a retrograde tract tracing method) into this neopallial zone. Neuron cell bodies filled with HRP reaction product were found clustered tightly in thalamus in an area, which in rat, corresponds to the medial part of the ventromedial nucleus.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: In a preceding study we found that the lateral olfactory tract in the opossum projects to the neocortical part of the insular cortex (suprarhinal cortex). The purpose of this present study is to demonstrate which thalamic cells, if any, project to suprarhinal cortex.

Methods Employed: The suprarhinal cortex of opossum was injected with the retrograde-transport-labelling enzyme, horseradish peroxidase (HRP), a method which demonstrates the location of neurons having axons terminating in the injection zone. At the time of injection of the HRP, the olfactory bulb on the same side of the injection was removed to elicit degeneration of the terminals of the olfactory bulb axons. Neuron cell bodies containing HRP were identified by treating the brain sections with tetramethyl benzidine (a non-carcinogen, preferred over the popularly used diaminobenzidine, DAB). The presence of degenerating terminals (detected with the cupric-silver method of de Olmos) in the injection zone served to verify that the HRP injections were in the olfactory projection area. Alternate brain sections were treated for the HRP reaction or with the cupric-silver method of de Olmos. Preliminary material has been successful enough to warrant further use of this combination.

Major Findings: Cell bodies containing HRP reaction product were found as a tight aggregate in the medial part of the ventromedial thalamus following HRP injections in the suprarhinal cortex. No cell bodies were found in mediodorsal thalamus.

Significance to Biomedical Research and the Program of the Institute: The route of olfactory input to frontal neocortex in rat has recently been shown to be: olfactory bulb to the piriform (olfactory) cortex to mediodorsal thalamus and to neocortex. In opossum, evidence has been given by Switzer and Heimer for a direct path from the olfactory bulb to suprarhinal cortex. The data in this report show thalamic neurons which project to this area in opossum are not located in mediodorsal thalamus, but are tightly grouped in a medial part of ventromedial nucleus. However, the neocortical representation for gustation in opossum is adjacent to the neocortical olfactory projection zone; therefore further injections are necessary using the combined technique of cupric-silver and HRP to determine if (1) our injections of HRP infringed on the cortical area for gustation; (2) there is an overlap of gustatory and olfactory representation in cortex and/or thalamus.

Proposed Course: To be continued.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00873-02 LBEB

PERIOD COVERED
October 1, 1977 through September 30, 1978

TITLE OF PROJECT (80 characters or less)
A Taxonomic Classification of Mammals Based on the Trajectory of Dorsal Lateral Olfactory Tract

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R.C. Switzer, III	Senior Staff Fellow	LBEB NIMH
Other:	J.M. Hill	Biologist	LBEB NIMH

COOPERATING UNITS (if any)
J.I. Johnson, Professor, Zoology, Psychology, Biophysics, Michigan State University; J. Kirsch, Professor, Peabody Museum, Yale University

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.6	.3	.3

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A review of the olfactory formation in over 100 mammals revealed that in some species the dorsal component of the lateral olfactory tract (dLOT) runs above the internal granule (IG) cells of the accessory olfactory formation, while in others the tract runs below the IG cells. The initial analysis of grouping the mammals according to the position of the dLOT demonstrated a division among the placental mammals that corresponded to that based on fossil evidence. Recently, specimens from three orders of placentals (elephant shrew, bat and pika) became available which had not yet been examined for this feature, thus affording a more complete data base. In all of these new specimens the dLOT passes through the accessory olfactory formation; thus they are to be grouped with rodents, primates and insectivores. Beyond the value to taxonomy, these data permit the generation of hypotheses concerning the temporal dynamics of ontogeny.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: Our initial observations of some placental and marsupial mammals allowed us to hypothesize that the position of the dorsal lateral olfactory (dLOT) could be used as a criterion in dividing the placental mammals into two distinct groups. In hoofed animals and carnivores (animals derived from leptictid-like insectivoran ancestors), the dLOT passes below the granule cells of the accessory olfactory formation (AOF). In the other placental mammals which include rodents, primates, lagomorphs, and edentates (thought to be derived from paleoryctid-like insectivores), the dLOT passes through the AOF between the large output neurons and the more deeply situated granule cells. This year other brain specimens became available which allowed a more thorough and almost complete sampling of the latter group. The specimens examined are from the orders: (i) Chiroptera (bat), (ii) Macroscelida (elephant shrew), (iii) Lagomorpha (pika).

Methods Employed: Brains were perfused and fixed with formalin, embedded in celloidin, sectioned at 30μ and stained for Nissl substance or myelin.

Major Findings: The new specimens, bat, pika, and elephant shrew exhibit the dLOT passing through the accessory olfactory formation. This evidence is particularly helpful to the taxonomy of the bat since its fossil record is poor.

From the analysis of this more complete sampling of the mammalian orders, the following conclusions are offered: (1) Since the position of the dLOT is below the granule cells of the AOF in monotremes and marsupials, it is considered to be the primitive or plesiomorphic trait. (2) The tract position in monotremes and marsupials is remarkably constant, reinforcing their status as major mammalian groups. (3) Other placentals, Hyracoidea, Perissodactyla, Artiodactyla and Carnivora appear to form a related group sharing the plesiomorphic trait. In this group the trait may be secondarily derived, or a reversion to or a maintenance of, the primitive trait. (4) Among placental mammals the Chiroptera, Dermoptera, Rodentia, Insectivora, Primates and Macroscelidea also appear to be related groups of possibly common ancestry sharing the derived (apomorphous) trait of the dLOT running through the AOF. (5) The Lagomorpha and Edentata do not share the genetic invariance or presumed uniform ontogenetic chronology of either of the larger groups and thus may be considered likely candidates for ancestry separate from that of either of the large placental assemblages.

Significance to Biomedical Research and the Program of the Institute: While these data are of particular value to taxonomy, it is also important to understand the cause of this dichotomy of position of the dLOT. Ontogenetic studies of a mouse suggest that the position of the dLOT is governed by precise sequential ordering of cell body migration (internal granule cells) and axon growth (from the output neurons of the main olfactory bulb).

It is not known what events caused an altering of these sequences which led to different final states of the neuron assembly of the mammalian groups. It is, perhaps, the realization of how easily the dLOT position can be altered by appropriate acceleration or retardation of axon growth and cell body migration that makes the invariance of the position of the dLOT within each group particularly remarkable. It is evident that ontogenetic timing of sequences has the potential of exerting a powerful and stable influence on evolution. The concept is best contained in a term recently popularized by S.J. Gould, heterochrony--changes in the relative time of appearance and rate of development of characters already present in ancestors.

Proposed Course: The data and analyses are in final draft form and are to be submitted for publication.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00874-02 LBEB

PERIOD COVERED
October 1, 1977 through September 30, 1978

TITLE OF PROJECT (80 characters or less)
The Neuroethology of Reproductive Behavior: Question of Role of Endogenous Opiates

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M.R. Murphy	Senior Staff Fellow	LBEB NIMH
Other:	S.C. Hamilton	Biologist	LBEB NIMH

COOPERATING UNITS (if any)
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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.3	.1	.2

CHECK APPROPRIATE BOX(ES)
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Opiate peptides and opiate receptors are concentrated in parts of the limbic system and striatal complex. It has been suggested that endogenous opiates may be involved in sexual behavior. This possibility was tested by observing the effects of the opiate antagonist naltrexone on the mating behavior of male hamsters. A detailed ethological analysis revealed several aberrations in mating behavior of naltrexone-treated animals. These deficits were more severe in animals receiving 20.0 mg/kg of naltrexone than in animals receiving 2.0 mg/kg. In general, their behavior appeared sluggish and inattentive. They were capable, however, of successfully impregnating females. Animals receiving 200.0 mg/kg naltrexone showed severely impaired copulatory behavior, but did not appear distressed, ataxic, or somnolent. In a second experiment animals were sacrificed at various stages of mating behavior and their brains assayed for opiate receptor activity; no differences were found. In a third experiment, continous administration of naltrexone by slow release pellets decreased fertility in female hamsters and survivorship of their pups.

Project Description:

Objectives: The purpose of this project is to identify forebrain mechanisms of species-typical reproductive behavior. The suggestion that the endogenous opiates may be involved in reproductive behavior comes from two areas: (1) Opiate peptides and opiate receptors are found in parts of the limbic system known to be involved in copulation; (2) Clinical evidence suggests that opiate drugs affect the sexual behavior of human beings. In the current experiments the effects of administration of a potent blocker of opiate receptors were examined with respect to copulation in males and fertility and maternal behavior in females. Also, an attempt was made to determine if endogenous opiates are released as a function of mating activity.

Methods Employed: Experiment I. For the purpose of comparing the effects of smaller and larger doses of naltrexone with the known changes following 20.0 mg/kg (see Progress Report No. Z01 MH 00874-01 LBEB), ten sexually experienced male hamsters were tested once after an injection of 2.0 mg/kg of the opiate antagonist and once after an injection of physiological saline; ten other males were tested after an injection of 200 mg/kg. Sixteen behavioral patterns relating to mating were recorded on a computerized event recorder and analyzed with respect to frequency, total duration, latency, and average duration of each activity. Experiment II. Nine female hamsters were implanted subcutaneously with pellets of naltrexone which provided slow release of the drug at approximately 3.5 mg/kg per day for thirty days. Experiment III. Groups of six male hamsters each were sacrificed after (1) no mating; (2) achieving ejaculation; (3) mating to exhaustion; and (4) mating to exhaustion and then 15 minutes rest. The brains were immediately removed and frozen for later radioassay of endorphins; blood plasma was obtained and frozen for the same purpose.

Major Findings: Experiment I. It was previously reported that administration of 20.0 mg/kg of naltrexone resulted in a mating deficit characterized by an increased latency to initiate copulation, a decreased frequency and duration of several mating activities, and an increased average duration of several behaviors. The mating behavior of such drugged males was described as "sluggish and inattentive." The mating deficit obtained after 2.0 mg/kg of naltrexone was not as severe as that after 20.0 mg/kg, but was generally similar: while there were almost no differences in the behavior of the control groups in the two experiments, the animals receiving 20.0 or 2.0 mg/kg of naltrexone differed significantly on 12 behavioral measures; all differences were in the direction of decreased effect in the animals receiving the smaller dose. The mating behavior of male hamsters receiving 200.0 mg/kg of naltrexone was severely impaired, particularly in the second half of the 30 minute test. Even with this massive dose of naltrexone hamsters did not appear distressed, ataxic, or somnolent and 7 out of 10 males performed some mating.

Experiment II. While 8 of 9 control females delivered pups, only 5 of 9 females implanted with naltrexone did so. The mean litter size for the two groups was about the same at birth, but 3 of 5 lactating females that received naltrexone lost pups before weaning, resulting in a mean reduction in pups of 36 percent. Only one control pup died, an overall decrease of only 3 percent. As a combined result of the decreased fertility and loss of pups, only 3 of 9 impregnated females implanted with nalrexone delivered and reared 3 or more pups to weaning, whereas 8 of 9 control females did so ($p < .025$, Fisher's Exact Test, one-tailed). The growth curves of surviving pups in experimental and control litters were not significantly different.

Experiment III. Thus far no differences in opiate receptor activity in the brains of animals in different stages of sexual excitement have been found. The analysis of blood endorphin levels has not been completed.

Significance to Biomedical Research and the Program of the Institute: The results of the experiments reported here do not indicate a major role for the endogenous opiate system in the control of reproductive behavior. Even with the massive dose of 200 mg/kg naltrexone, 70 percent of males performed some mating. The changes in behavior observed following administration of naltrexone could be general behavioral effects, not specific to mating. The possibility that the administration of opiate antagonists results in a decrease in pup survival needs further analysis to determine if abnormal maternal care is at fault.

Proposed Course: To be continued.

Publications: None.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00875-01 LBEB

PERIOD COVERED
October 1, 1977 through September 30, 1978

TITLE OF PROJECT (80 characters or less)
Identification of Forebrain Units that Discharge in Phase with Respiration

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	P.D. MacLean	Chief, LBEB	LBEB NIMH
	A.H. Orr	Research Psychologist	LBEB NIMH
	M. Eckardt	Research Psychologist	LBEB NIMH
Other:	R.M. Sewell	Biologist	LBEB NIMH

COOPERATING UNITS (if any)
Richard Radna, Dept. of Neurosurgery, Mt. Sinai Hospital, New York, New York
Michael Walsh, Section on Technical Development, NIMH

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NIMH, ADAMHA, NIH, Poolesville, Maryland 20837

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.3	1.5	.8

CHECK APPROPRIATE BOX(ES)
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In an extension and confirmation of the findings reported in Project No. Z01 MH 00860-03 LBEB, units have been identified in parts of the limbic system and corpus striatum that discharge in association with repiration. In addition, similar units have been found in neocortical areas. Of 204 systematically tested units, 12 percent could be characterized as the respiratory type.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: In a preceding microelectrode study in awake sitting squirrel monkeys, there were indications that vagal volleys entrained units in certain parts of the forebrain to discharge in association with respiration. Such units were found in the region of the limen insulae and in certain parts of the amygdala, hippocampus, and putamen. The purpose of the present study is to learn whether or not there are units in the forebrain that spontaneously discharge in phase with respiration and to obtain information about the relative incidence of such units in various structures.

Methods Employed: The stereotaxic device employed for exploring the brain with microelectrodes in gently restrained, awake sitting squirrel monkeys has been described in previous reports. For extracellular recording, glass micropipettes are used that have a DC resistance of five to 10 megohms. A small thermistor placed near the left nostril of the monkey, provides a means of obtaining a stable recording of the spirogram and triggering the oscilloscope. During an experiment both the spirogram and the unit discharge are displayed on an oscilloscope. Recordings of unit activity, the spirogram, the EKG, and other measures are permanently stored on magnetic tape which allows a retrospective computer-assisted analysis of the data (see Z01 MH 00858-01 LBEB). The recording system also provides on-line analysis.

Major Findings: In an extension and confirmation of last year's findings, units have been isolated in the claustrum, hippocampus, and corpus striatum that discharge in association with respiration. The cyclical discharge of a number of these units is clearly evident by the sound of their discharge and by visual inspection of the time-interval histogram. With the aid of computer-assisted analysis, a number of units in the supracingulate cortex and dorsolateral cortex of the frontal lobe have also been found to discharge in association with respiration. Of 204 systematically tested units, 12 percent discharged in association with respiration. There were 4 such units in the hippocampus; 2 in the claustrum; 1 in the entorhinal cortex; 5 in the putamen; 1 in the caudate; 4 in the supracingulate cortex; 5 in the frontal cortex of the outer convexity; and 3 in the pregeniculate nucleus. More experiments will be required to find out the relative incidence of such units in different structures.

Significance to Biomedical Research and the Program of the Institute: The discovery that units of the forebrain discharge in association with respiration has implications reaching back to the Greek use of the word psyche--meaning breath--to refer to the mind or spirit. The findings raise such questions as: (i) What is the possible role of such units in contributing to the central excitatory state required for consciousness? (ii) How is the activity of such units affected during sleep, as well as during dreaming when there may be marked cardiorespiratory changes? (iii) What is the possible role of such units in psychological functions such as recognition ("breath of recognition"); motivation (e.g., sudden change of respiration

in feats requiring unusual strength); emotional states (e.g., startle, joy, depression, etc.,); memory and creative thought ("inspiration"). (iv) How is the activity of such units possibly related to the development of hypnotic or hypnagogic states?

Proposed Course: To be continued.

Publications:

Radna, R.J. and Vaughn, W.J.: Computer-assisted unit data acquisition/reduction. Electroenceph. Clin. Neurophysiol., 44: 239-242, 1978.

Poletti, C.E. and Creswell, G.: Fornix system efferent projections in the squirrel monkey: An experimental degeneration study. Journal of Comparative Neurology, 175: 101-128, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00876-01 LBEB

PERIOD COVERED
October 1, 1977 through September 30, 1978

TITLE OF PROJECT (80 characters or less)
An Attempt to Explain Occasional Deficits in Display Behavior of Squirrel Monkeys Following Ablations of Prefrontal Cortex

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	P.D. MacLean	Chief, LBEB	LBEB NIMH
Other:	R.E. Gelhard	Biologist	LBEB NIMH

COOPERATING UNITS (if any)
NONE

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.45	.05	.4

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of the present study was to learn whether or not complicating changes in the caudate nucleus might account for inconsistent findings on the effects of prefrontal ablations on species-typical displays of gothic-type squirrel monkeys. Volumetric measurements of the corpus striatum revealed that a reduction in mass of the caudate nucleus may occur as a complication of surgical entrance of the ventricles. Such a complication may have contributed to the deficit in one of two monkeys that failed in the performance of displays following prefrontal ablations.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: In a preceding study, different areas of the prefrontal and orbital cortex were ablated for the purpose of observing the effects of such lesions on the species-typical mirror display of gothic-type squirrel monkeys. Two of seven animals showed a persisting, statistically significant deficit in performing displays. The discrepant outcome in these experiments could not be explained by differences in the location of the frontal lesions or the extent of retrograde degeneration in the medial dorsal nucleus. In both of the positive cases, the ventricles had been entered, and there was some dilatation of the anterior horns as well as flattening of the head of the caudate nucleus. Since it has been shown in other experiments that the striatal complex is implicated in display behavior, it was the purpose of the present study to learn whether or not the behavioral deficits in the two animals in question could be correlated with loss of neural substance in the caudate nucleus.

Methods Employed: Reference should be made to an accompanying progress report for a description of the mirror display test used in this investigation (see Z01 MH 00851-14 LBEB). The prefrontal ablations in seven squirrel monkeys (H-3, I-3, J-3, K-3, N-3, R-4, S-4) was accomplished by subpial aspiration of the cortex. In these seven operated animals and two controls, volumetric measurements of the caudate nucleus and putamen were obtained by planimetry according to the method of Dornfeld et al (1942).

Major Findings: All operated animals were followed for a period of three to six months (X=3.3), allowing sufficient time for retrograde degeneration in N. medialis dorsalis. In addition to the two animals with behavioral deficits (H-3, J-3), the ventricles were entered in four other cases (I-3, K-3, N-3, S-4), and there was slight to moderate dilatation of the anterior horns. There was a remarkable consistency in the ratio of the volumes of the caudate and putamen in all cases except H-3. Only in monkey H-3, one of the two animals with a persisting behavioral deficit, was there a significant deviation from the mean. This animal was also distinctive in showing the greatest amount of dilatation of the anterior horns and the most extensive flattening of the head of the caudate nucleus.

Significance to Biomedical Research and the Program of the Institute: In animal experimentation on the frontal lobes, it seems to be recognized, but seldom stated in writing, that prefrontal ablations may involve entrance into the ventricles and be complicated by some dilatation of the anterior horns and an apparent diminution in the mass of the caudate nucleus. It appears to be unlikely that severance of the prefrontal connections with the caudate nucleus would account for a measurable shrinkage of its mass. Volumetric measurements in the present study indicate that a measurable reduction in the caudate nucleus may occur as a complication when the ventricle is entered. Such a complication may have contributed to the behavioral deficit in one of the two atypical cases in the present study.

Proposed Course: Concluded.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do **NOT** use this space)

U.S. DEPARTMENT OF
HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00877-01 LBEB

PERIOD COVERED

October 1, 1977 through September 30, 1978

TITLE OF PROJECT (80 characters or less)

Effects of Apomorphine on Behavior of Domestic Turkeys

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	P.D. MacLean	Chief, LBEB	LBEB NIMH
Other:	R.E. Gelhard	Biologist	LBEB NIMH

COOPERATING UNITS (if any)

NONE

LAB/BRANCH

Laboratory of Brain Evolution and Behavior

SECTION

INSTITUTE AND LOCATION

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.45	.05	.4

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The striatal complex constitutes a fundamental part of the forebrain in reptiles, birds, and mammals. Neurochemical studies have shown that parts of the striatal complex are rich in dopamine. As part of a comparative investigation on the functions of the striatal complex, tom turkeys were injected with apomorphine which is presumed to excite dopamine receptors. A dosage was found that regularly resulted in a syndrome characterized by relaxation of the snood, headshaking, pseudo-food-searching, pecking, separation from the flock, and continuous locomotion. With the occurrence of darkness, there was a cessation of locomotion, but not the initial symptoms of snood relaxation and headshaking.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: One of the values of comparative studies on submammalian forms is that they may provide leads regarding the function of structures of the mammalian brain. The work of this Laboratory is particularly devoted to elucidating functions of the striatal complex which is a fundamental part of the forebrain in all terrestrial vertebrates. Advances in neurochemistry have helped to identify the corresponding structures in the brains of reptiles, birds, and mammals. Part of the striatal complex is rich in dopamine which derives from dopaminergic cells in the midbrain. The purpose of the present project is to assess in turkeys the action of the apomorphine which is presumed to have an excitatory effect on dopamine receptors. Of avian forms, turkeys (Meleagris gallopavo) are particularly well suited for observations on species-typical forms of behavior, as well as associated autonomic changes reflected in the wattles and snood.

Methods Employed: Subjects are tom turkeys maintained in a large outdoor habitat (20-foot radius) surrounded by an opaque wall of 8-foot filon sunk two feet into the ground. The behavioral characteristics of each animal with respect to the strutting display, group gobbling, etc., are well-known on the basis of year-long observations. Apomorphine is administered by intramuscular injection in doses ranging from 0.01-1.0 mg/kg.

Major Findings: Doses of 0.2 mg/kg elicited most of the manifestations observed with doses ranging from 0.015 mg/kg to 1 mg/kg. The apomorphine syndrome includes relaxation of the snood, headshaking, pseudo-food-searching pecking, separation from the flock, and continuous locomotion. Retching and salivation occur inconsistently and unpredictably. The first sign usually to appear is relaxation of the snood, occurring within 1 to 4 min ($\bar{X}$, 2.3 min). The snood is completely relaxed by 6 min and may remain so for as long as six hours. The minimum effective dose for snood relaxation was 0.025 mg/kg. Headshaking begins to appear shortly after the relaxation of the snood and may be performed so intensively as to sound like a cracking whip. It, too, may persist into the 6th hour. With doses of 1 mg/kg, young turkeys may engage in rapid running in and out of the flock for periods of up to three hours. Treated animals do not engage in the strutting display or in group gobbling.

A finding of unusual interest was the cessation of locomotion after darkness. Animals injected during darkness showed the relaxation of the snood and headshaking, but even with doses of 1 mg/kg, did not engage in locomotion. The absence of the locomotor response under these conditions was not dependent on a diurnal rhythm, since the same result was obtained when an animal was injected in a darkened room during the middle of the day.

Significance to Biomedical Research and the Program of the Institute: The present experiments suggest that the turkey may prove to be a useful animal for testing the interaction of neuropharmacological agents affecting dopamine receptors. A finding of considerable interest was the profound

inhibiting effect of darkness on apomorphine-induced locomotor activity. Since the nucleus opticus tegmenti is wedged between the groups of dopaminergic neurons (A10 and A9) that innervate the olfactostriatum and corpus striatum, the findings suggest that the accessory optic system may be implicated in the darkness effect.

Proposed Course: To be continued.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00878-01 LBEB

PERIOD COVERED
October 1, 1977 through September 30, 1978

TITLE OF PROJECT (80 characters or less)
Computer-Based Recording and Analysis of Species-Typical Behavior

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: M.R. Murphy Senior Staff Fellow LBEB NIMH

COOPERATING UNITS (if any)
None

LAB/BRANCH
Laboratory of Brain Evolution and Behavior

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Poolesville, MD 20837

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.1	.1	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A highly accurate and flexible computer-based system for recording and analyzing species-typical behavior has been developed. This system is currently being applied to research on neural and endocrine mechanisms of reproductive and aggressive behavior. Data collected using this system provide a highly reliable frame of reference for measuring the effects of brain lesions, drugs, and other manipulations on complex behavioral patterns.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: The purpose of this project is to develop improved methods for describing, recording, analyzing, and comparing species-typical behavior.

Methods Employed: For making behavioral observations a standard computer terminal keyboard and small computer are used as an event recorder. The observer records the time of occurrence of various behaviors by depressing appropriate keys. Each observation is simultaneously displayed on a video screen for confirmation, and any errors of the observer may be corrected immediately. Observations may be made of live animals or of videotaped behavior. If slow or fast motion videotape is used, the computer can convert the data to real time. At the end of an observation period a permanent record of the data is stored on a magnetic disk. Special computer programs provide for immediate write-out of the raw data and the computation of summaries such as means and standard deviations. Each observation period is analyzed with respect to the latency, frequency, total duration, and average duration of each type of behavior performed. Other programs provide for the combination of individual records into experimental groups and for the computation of group summaries and statistical comparisons between groups.

This system is currently applied to research on neural and endocrine mechanisms of reproductive and aggressive behavior in hamsters.

Major Findings: This system is faster, more accurate, more flexible and has a larger capacity than conventional methods of recording and analyzing species-typical behavior. The original data record contains complete information regarding the frequencies, latencies, durations and sequential relationships of the behaviors observed. This original record is maintained permanently and may be reanalyzed conveniently, in whole or part, using different statistical or mathematical procedures.

An illustration of the utility of this data recording method is found in the analysis of the effect of the opiate antagonist naltrexone on the mating behavior of male hamsters (see Progress Report No. Z01 MH 00874-01 LBEB). Heretofore, conventional forms of testing had failed to show any notable behavioral effect of this potent blocker of opiate receptors. With the system under discussion, the duration of each diagnostic feature of mating was recorded. The computer-assisted analysis revealed that naltrexone had selectively reduced the occurrences of short bouts of certain behaviors without affecting the frequency of longer bouts.

Significance to Biomedical Research and the Program of the Institute: In the analysis of neural mechanisms of behavior the need for improved techniques of studying and manipulating the nervous system is obvious. It is not so obvious that improved techniques for recording and analyzing behavior are just as essential. The quantitative methods described in this

report provide a highly reliable frame of reference for measuring the effects of brain lesions, drugs, and other manipulations on complex species-typical behavior.

Proposed Course: To be continued.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00879-01 LBEB

PERIOD COVERED

October 1, 1977 through September 30, 1978

TITLE OF PROJECT (80 characters or less)

Trigeminal Input to the Cerebellar Tactile Areas

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R.C. Switzer, III	Senior Staff Fellow	LBEB NIMH
	C.R.R. Watson	Visiting Scientist	LBEB NIMH

COOPERATING UNITS (if any)

NONE

LAB/BRANCH

Laboratory of Brain Evolution and Behavior

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Poolesville, Maryland 20837

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.4	.3	.1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Recently, five tactile representations were demonstrated in the cerebellar cortex of the rat by Shambes et al. These five separate representations cover a large area of cerebellum and are chiefly devoted to the face and mouth. The different organization of each suggests that they have individual significance in certain behavioral sequences. The anatomical connections are not known. In the present study, the horseradish peroxidase-retrograde tracing technique was used which allows the identification of distant nerve cell bodies with axons terminating in the cerebellum. The results demonstrate that the trigeminal input to cerebellum comes only from the principal and interpolar nuclei. In addition, it appears that the trigeminal input to cerebellum, particularly the hemisphere, is much more extensive than previously realized. These data suggest that the trigemino-cerebellar system will be found to play a major role in facial and oral behavioral sequences.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: Electrophysiological studies have demonstrated short latency responses in the cerebellum elicited by tactile stimulation of facial areas in rat. Since it seemed likely that these cerebellar responses arose directly from the brain stem trigeminal nuclei, proof of a direct connection was sought by using horseradish peroxidase (HRP) as a retrograde transport labelling technique.

Methods Employed: Injections of horseradish peroxidase were made in areas of cerebellum known to respond to tactile stimulation of the face. Sections of the brain stem were treated with a new, more sensitive chromagen for HRP, tetramethyl benzidine.

Major Findings: The approximate distribution of cells filled with HRP reaction product in the trigeminal nuclei are proportionately shown by x's in the following table:

Trigeminal nuclei	Left (Injected Side)	Right (10 x's=maximum)
Mesencephalic	0	0
Principal	xxx	x
Oralis	0	0
Interpolaris	xxxxxxx	xxx
Caudalis	0	0

Significance to Biomedical Research and the Program of the Institute: Recent studies have demonstrated a greater role for cerebellum than the classical proprioceptive 'organ'. Our data in this report indicate that rat cerebellum gets direct input from the trigeminal nucleus meaning that the cerebellum receives tactile information before it is received by neocortical areas. It can therefore be inferred that tactile 'feedback' to cerebellum may play a greater role in fine motor activity than the input derived from muscle spindles (proprioceptive input). From the broader perspective of the modulatory role of cerebellum on the striatum (dopamine levels and neuron activity), our data provides evidence of how exteroceptive input (body surface stimulation) has access to striatum by a previously unsuspected route.

Proposed Course: To be continued.

Publications:

Watson, C.R.R. and Switzer, R.C. III: Trigeminal projections to cerebellar tactile areas in the rat - Origin mainly from nucleus interpolaris and nucleus principalis. Neuroscience Letters (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00880-01 LBEB

PERIOD COVERED

October 1, 1977 through September 30, 1978

TITLE OF PROJECT (80 characters or less)

Question of Direct Connections Between the Brain and Pineal Body in Rat

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J.C. Harris	Guest Worker	LBEB NIMH
	R.C. Switzer, III	Senior Staff Fellow	LBEB NIMH

COOPERATING UNITS (if any)

NONE

LAB/BRANCH

Laboratory of Brain Evolution and Behavior

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Poolesville, Maryland 20837

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.25	.05	.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In the ferret and a few other animals, there are, according to some reports, direct connections between the brain and the pineal body. No such connections have been demonstrated in the rat. In the present study, a sensitive horseradish peroxidase method is being used as a further means of learning whether or not any cerebral neurons project to the pineal body. The findings thus far have been negative.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: The long evolutionary history of the pineal body would suggest that it plays some important role in cerebral function and behavior. In this laboratory, comparative studies are being conducted on the anatomy and functions of this gland (see Progress Report Nos. Z01 MH 00841-01 and Z01 MH 00842-01). In the ferret and a few other animals, there are, according to some reports, direct connections between the brain and pineal body. No such connections have been found in the rat. In the present study, a sensitive horseradish peroxidase method is being used as an additional means to learn whether or not any cerebral neurons project to the pineal body.

Methods Employed: A hydrogen peroxidase method utilizing tetramethyl benzidine is being employed in the present experiments. A neurosurgical approach has been devised for injecting the agent into the pineal body of rats under direct vision.

Major Findings: The pineal body has been successfully injected with HRP, but thus far no HRP-staining neurons have been found in representative sections of the upper and lower brainstem.

Significance to Biomedical Research and the Program of the Institute: The present study may be expected to provide basic anatomical information that is requisite for studies attempting to elucidate the functions of the pineal gland.

Proposed Course: To be continued.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00834-02 LBEB

PERIOD COVERED

October 1, 1977 through September 30, 1978

TITLE OF PROJECT (80 characters or less)

Social Group Organization

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. L. Hill	Visiting Associate	LBEB NIMH
Other:	J. H. Burkholder	Guest Worker	LBEB NIMH

COOPERATING UNITS (if any)

none

LAB/BRANCH

Laboratory of Brain Evoluiton and Behavior

SECTION

Unit for Research on Behavioral Systems

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Poolesville, Maryland 20837

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.9	0.8	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Examination is being made of the social/spatial organization of a freely growing population of confined house mice. Mathematical and statistical techniques are being utilized to analyze group structure in time and space. Particular emphasis is being placed upon determining those factors which are related to social group development during the early phases of population growth. It is essential to know if early changes in the characteristics of social/spatial organization are related to the cessation of reproduction and thus the eventual decline to extinction of high density populations.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: To determine the principles of group organization, structure, and intragroup communication which operate to foster development and maintenance of optimal-sized groups within (i) growing, (ii) stable, (iii) overcrowded, and (iv) declining populations. Social roles of individuals within groups, as well as the contribution of group stability to population survival, are being investigated.

Methods Employed: Cluster analytic techniques, including varimax rotation were used to develop factor-loadings or weighting of association scores in the large matrices analyzed. The data analyzed by these methods were the inter-animal distances between diurnal resting site locations by all animals in freely-growing laboratory populations of house mice during each of fourteen censuses conducted during the early phase of population growth. Animals were assigned to groups on the basis of their degree of association with the mathematical factors identified by these procedures.

Major Findings: Preliminary analyses indicate that the groups changed in number, membership, sex composition, location, size, distance apart, and in internal spatial organization during the initial seven months of the study. During the final two to three months of the study the groups became relatively stable in membership and location. This stable social organization was most clearly seen in the largest, fastest growing population in which several social groups coalesced into four large groups. This collapse in the social organization apparently preceded a decrease in the rate of population increase.

Significance to Biomedical Research and the Program of the Institute: Many animals, including human beings are adapted to develop and live most effectively within small, relatively stable, social groups. However, urban induced anonymity, frequent change of residence, and crowding reduce the probability that meaningful interactions will occur at the appropriate intensity and frequency. Our studies of the principles of group organization among rodents contribute to the understanding about requirements of group living. Our results suggest that normal social behavior in mice, as measured by rate of reproduction, is most closely related to social group size. When the social group size is approximately ten animals or less, although membership and location of such groups may change, a high rate of reproduction is maintained. As population density increases the animals are no longer able to distribute themselves into small social groups and the resultant increase in group size apparently precipitates a breakdown in normal social behavior as indicated by an eventual decrease in reproductive rate.

Proposed Course: Further analyses on the movement of individuals, and of each cohort born into the populations, are expected to reveal processes whereby optimum group size is attained and what factors lead to the eventual breakdown of social organization as optimal group size is surpassed in overcrowded populations.

Publications:

Hill, James L.: Space utilization of Peromyscus: Social and Spatial Factors. Animal Behaviour, 25: 373-389, 1977.

Burkholder, J. H.: An interactive version of MULR04 with enhanced graphics capability. Multiple Linear Regression Viewpoints, 8:22-44, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00835-02 LBEB

PERIOD COVERED
October 1, 1977 through September 30, 1978

TITLE OF PROJECT (80 characters or less)

Crowding and Population Dynamics

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. B. Calhoun	Research Psychologist	LBEB	NIMH
Other:	J. L. Hill	Visiting Associate	LBEB	NIMH

COOPERATING UNITS (if any)

none

LAB/BRANCH
Laboratory of Brain Evoluiton and Behavior

SECTION
Unit for Research on Behavioral Systems

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH Poolesville, Maryland 20837

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.0	.8	2.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project deals with phenomena characterizing a house mouse population as it proceeds through successive stages of crowding resulting from several population doublings in an eight cell, experimentally-designed habitat presumed optimum for 100 mice. From the initial 16 colonizers the population has been permitted to increase each 200 days from 16 to 100 to 200 to 400 to 800 mice. This year's report concentrates on the establishment of adult social groups during the 60 days out of each 200 days when progeny were permitted to remain in the population to form the next generation. The design of the habitat represents a major aspect of this study. It is an octagon, 16 feet across, with metal walls. Major resources such as nesting boxes, food and water are located on the walls; the mice gain access to them by climbing vertical wire ramps. Low radial barriers, over which the mice can climb, divide the habitat into 8 pie-shaped cells. Lower barriers divide each cell into 2 subcells such that the universe may be considered to consist of 16 subcells. The counter clockwise subcells are apparently preferred because their nest boxes are one-half the height of those in clockwise subcells, and less energy expenditure is required to move between the nest boxes and other resources.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: To determine how the physical structure of the environment, and the behavioral and social history of members of the population, influence composition and stability of social groups.

Methods Employed: All procedures of handling the mice and making observations follow a 200 day duration recurrent cycle. During the first 140 days of the cycle all progeny born during this 140 days are removed before they in turn produce progeny. This tactic provides adults with experience in rearing young. Post-reproductive adults are then removed from the population. The final 60 days of each 200 day cycle is devoted to establishing the next generation. Enough young are permitted to survive to double the entire population from the number of adults present before removal of the post-reproductive individuals. By this strategy, each successive generation is produced by experienced prime reproductive aged survivors of the two prior generations. Identification of each mouse is accomplished prior to weaning by the attachment of a pair of numbered metal ear tags. In a predetermined census schedule all mice are captured during the least active phase of their daily activity cycle. During this time these nocturnal animals are associated with their preferred diurnal resting residences. Analyses of these resting residence data are, thus far, restricted to the two 60 day breeding periods approaching the end of the first 400 and 600 days since initiation of the population. Nine to 12 residences were recorded for each mouse during each breeding period.

Major Findings: By 200 days of age approximately 90% of the residence records of each mouse were associated with only one of the 16 subcells. This fixation of residence produced a remarkable stability of breeding group membership. Location of an individual's adult or breeding group residence was randomly distributed with respect to the location of its birth. Breeding females, and associated dominant and territorial males, were found in the highest proportion in the counter clockwise subcell in each cell. This may have resulted because this subcell has lesser demands on locomotor energy expenditure in moving between nesting boxes and other resources than does the clockwise subcell. Severly injured and other socially low ranking males were most frequently found in the clockwise subcell locations. Further, social structuring of the total environment occurred: One-half of the universe, comprised of contiguous cells 1, 2, 8 and 7 became inhabited by 40% more males than the other half of the universe, and contained 15% more mice totally.

Significance to Biomedical Research and the Program of the Institute: This demonstration that design of the physical environment can enhance group stability suggests that experimental manipulation of the physical environment of other animals may contribute to development of architectural design principles that may ameliorate social stress on the human level. The new principles of design we introduced in the current environment were more effective than we had anticipated. They sufficed to push the optimum density towards 200 adult mice rather than the anticipated 100, perhaps by creating more clearly demarcated subunits of the physical area within which individuals

could associate.

Proposed Course: We will continue following this population through further doublings at 200-day intervals until the ultimate stage of crowding is reached. This is a time when every member of a last generation is characterized by an asocial, non-reproducing, "autistic-like" state. We have hypothesized that this stage would have been reached by the time the fifth generation were adults and the total population reached 800 individuals. However, it now looks as though we will have to follow the history of this population for another 400 to 600 days until the 6th generation is in adulthood and the total population stands at near 1600.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 MH 00836-02 LBEB

PERIOD COVERED
October 1, 1977 through September 30, 1978

TITLE OF PROJECT (80 characters or less)
Conceptual Adaptation and Evolution

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. B. Calhoun	Research Psychologist	LBEB NIMH
Other:	H. E. Cascio	Electronics Engineer	DRS BEIB NIH
	L. E. Olson	Physicist	
	R. W. Ridel	Research Psychologist	LBEB NIMH
	M. J. Walsh	Electronics Engineer	STD NIMH

COOPERATING UNITS (if any)
BEIB, NIH
STD, NIMH

LAB/BRANCH
Laboratory of Brain Evolution and Behavior

SECTION
Unit for Research on Behavioral Systems

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH Poolesville, Maryland 20837

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.2	1.4	1.8

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

By utilizing various strains of rats, Rattus norvegicus, a paradigm of human culture is being explored. This paradigm is based on the following theoretical considerations: The evolution of species of mammals, including human beings, has resulted in optimum group sizes of 12 adults. Since each group occupies a fixed territory, eventual occupation of all available space by such groups culminates in a stable adult population of species. However, if conditions permit elaboration of social roles (particularly cooperative ones) and development of complex communication networks, then more individuals can survive in a given area without a resulting stressful increase in rate of interaction among members of the population that would otherwise trigger limitation of population size. This process which has permitted successive doubling of the human population is being experimentally simulated by providing computer controlled contingencies for rats living in a complex physical environment that fosters development of cooperative social roles and more complex communication networks.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: To determine if the enhancement of conceptual capacity by rats induced by acquisition of complex cooperative social behavior can ameliorate or prevent the origin of stress normally accompanying increase in density above the biologically determined optimum.

Methods Employed: As a result of our experience over the past two years major changes in our computer facilitated behavior recording and influencing system have been made. Furthermore, we have had to modify our strategy for maximizing population genetic diversity. Our first start of this study involved 2 strains of wild-type rats and 4 strains of domesticated ones. The marked divergence of these behavioral types of rats produced such social instability and stress that this study had to be discountinued. Our newly initiated study starts with double F_1 type rat subjects derived by first crossing each of two strains of wild type rats with a domesticated breed, and then these two different F_1s were crossed to produce the colonizers for the experiment. Conditions in each of two habitats provide the means for inducing cooperative intersex drinking and for simultaneous eating only by rats of similar social status. Such contingencies will be continuously activated for the experimental population. Subjects in both populations have continuous access to other large learning spaces where several additional situations continuously challenge cooperative and conceptual capacities.

Major Findings: None. The study was just initiated during September 1978 after a year of intensive developmental work. This development was made possible by intensive effort by members of the Section on Technical Development of the NIMH and by members of the Biomedical Engineering and Instrumentation Branch of the NIH.

Significance to Biomedical Research and the Program of the Institute: It is anticipated that at the end of this study the control rats will have experienced marked stress from crowding, will have ceased reproduction and become somewhat autistic-like, and will have exhibited little capacity to solve the cooperation demanding tasks available in adjoining learning space rooms. On the contrary the experimentals will exhibit few signs of social stress and will have solved many other collaborative tasks in the learning space rooms. If these anticipations prove correct, then our formulations of the paradigm of human culture will have been validated and insights derived therefrom may be applied to analogous situations in the human setting as the world population approaches a stage of extreme crowding. (See Z01 MH 00837-01 LBEB for vocalization research conducted on these populations.)

Proposed Course: Sixteen rats introduced into each habitat formed the colonizing population in the habitats deemed optimum for 12 adults. For the first 9 months the experimental population will successively be carried through simple drinking cooperation and then through training in intersex cooperation. At the end of this time a new generation of rats will be allowed to survive in each population, sufficient to bring the total to 48 in each. At this level the experimentals will be allowed to learn the social status,

conjoint eating habit. During the whole course of this study, until its completion at the end of 19 months, our technology will permit analysis of durations of individual and social behavior in all compartments of the environment.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00837-01 LBEB

PERIOD COVERED

October 1, 1977 through September 30, 1978

TITLE OF PROJECT (80 characters or less)

Cooperation and Crowding Induced Alterations in Vocalization by Rats.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. L. Hill	Visiting Associate	LBEB NIMH
Other:	J. H. Burkholder	Guest Worker	LBEB NIMH
	J. B. Calhoun	Research Psychologist	LBEB NIMH

COOPERATING UNITS (if any)

none

LAB/BRANCH

Laboratory of Brain Evolution and Behavior

SECTION

Unit for Research on Behavioral Systems

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Poolesville, Maryland 20837

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.6	0.4	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project is designed to determine if the frequency and character of rat vocalization changes as a function of social experience. Two populations of rats will be compared: One population will be trained to engage in cooperative behavior patterns to satisfy food and water requirements. The other population will serve as an experimental control group and will not be required to perform cooperative behaviors. It is expected that as each population triples in size those rats required to perform cooperative behaviors will exhibit a larger repertoire of vocal patterns than will the control group, while crowded control rats will exhibit vocalizations of diminished complexity.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: To determine if the quality, quantity and pattern of rat vocalizations changes when the animals are required to perform complex cooperative behavior patterns to meet their physiological needs.

Methods Employed: Vocalizations will be detected utilizing a microphone equipped with a parabolic reflector, and will be classified according to type and frequency utilizing the URBS PDP 11/04 computer and Tektronix 5L4N Spectrum Analyzer.

Major Findings: None

Significance to Biomedical Research and the Program of the Institute: Our hypothesis (see Proposed Course) is that crowding disrupts vocal communicative abilities and that the learning of cooperative social roles increases the complexity of vocal communication. In human evolution cooperative behavior may have had an important role in the development of verbal communication. The continued education of people in the performance of complex social behaviors may further enhance vocal communication. Enhancement of verbal communicative skills may reduce the effects of stress resulting from over-population.

Proposed Course: To monitor the vocalizations of the two rat populations (see Z01 MH 00836-02 LBEB) over the course of the next two years. Particular emphasis will be directed at the last 100 days of each successive 9-months with respect to the following hypothesis of alteration of vocalizations due to crowding and learning of cooperative behavior.

POPULATION TYPE

	1 CONTROL No opportunity for learning cooperative behavior.	2 EXPERIMENTAL Have opportunity for learning cooperative behavior.
Phase I N=16 1st 9 months.	1.1 (a) Vocalizations are of the normal wild type. (b) Rats experience only moderate stress from social living.	2.1 (a) Complexity of vocalization is increased over 1.1. (b) Very little social stress experienced. (c) Have had opportunity to learn intersex copperative drinking.
Phase II N=48 2nd 9 months.	1.2 (a) Vocalizations have become markedly fragmented and simplified. (b) Rats now at 4 times optimum density exhibit many signs of social stress.	2.2 (a) Vocalizations have increased even more in complexity. (b) No increase in social stress. (c) All rats have learned 3 types of cooperative behavior.

Summary expectation: Vocalization will exhibit an increasing complexity from 1.2 to 1.1 to 2.1 to 2.2.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00838-01 LBEB

PERIOD COVERED

October 1, 1977 through September 30, 1978

TITLE OF PROJECT (80 characters or less)

Theoretical Population Dynamics

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. B. Calhoun	Research Psychologist	LBEB NIMH
Other:	D. R. Ahuja	Post-doctoral FAES Fellow	LBEB NIMH
	G. J. Ross	Intergovernmental Exchange Fellow	LBEB NIMH

COOPERATING UNITS (if any)

none

LAB/BRANCH

Laboratory of Brain Evolution and Behavior

SECTION

Unit for Research on Behavioral Systems

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Poolesville, Maryland 20837

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.4	0.6	0.8

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

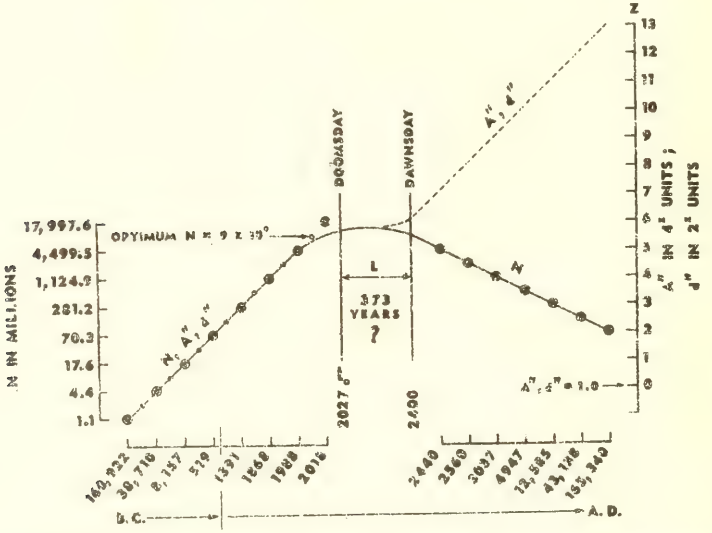
A model of world population is being derived. It includes a theory of the course of evolution which sees enhancement of awareness and responsiveness as the major central tendency. Preservation of this central tendency requires a gradual shift from an increasing to a decreasing human population. The change between these two trends has just begun. A maximum of more than eight billion people will be reached before decline in numbers begins. Certain traps or dangers will exist during the transition toward a pattern of decline in numbers. One of these traps results from an aging population: Our modeling from mouse and rat population studies indicates that a high probability of population extinction may occur when over 50% of the population consists of postreproductive individuals. The broader interpretation of this model holds that excessive numbers of post-reproductive or postproductive individuals inhibit acquistion by younger members of those social roles and other behaviors essential for species survival.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: To develop a theoretical world model of human population that will help the human species move through the several-hundred year crisis period ahead and then enter a new era of evolution that would permit the numbers of the human species to continue to increase their capacities for awareness and responsiveness for numerous further generations.

Methods Employed: Previously the generalized model diagrammed below was developed. Currently we are engaged in a collaborative effort with the United Nations Fund for Population Activities in which their world population data bank and computer programs are being utilized to estimate the shortest time in which this shift in change from an increasing to a decreasing population may ensue. Recent reassessment of the theory confirms earlier formulations, that is to say: A continuously declining population will have been fully established by 2442 A.D. (464 years from now), when a world population of four billion will have again been reached. One hundred and sixty-two leading behavioral scientists around the world have each prepared 1600-word statements about major researchable phenomena that may characterize this near-future relating to adaptation, environment, and population. These statements are being utilized to help construct models pertaining to the impact of these problems on population change and human welfare.



The sapient (left) and Szilardian (right) domains of human evolution. N = total world population. A" represents the total information available to any individual, while d" represents the codification of information into usable concepts.

Major Findings: We prefer to wait to make any further statement until the models are more fully developed.

Significance to Biomedical Research and the Program of the Institute: If we are correct in the general theory, as it is now taking form, the future health and social problems, as well as the character of research necessary to resolve these problems, will be quite different from that based on the simple ecological, wild-life carrying capacity model, which projects a continuing stable human population, forevermore, of between 6 and 12 billion persons.

Future Course: By the end of another year we should have made considerable advance in this general theoretical framework of long range population evolution.

Publication:

Ross, G. C.: A note on dynamics of population with history-dependent birth rate. Bull. of Math. Biol. 40: 123-131, 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do **NOT** use this space)

U.S. DEPARTMENT OF
HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00881-22 LCM

PERIOD COVERED
October 1, 1977 through September 30, 1978

TITLE OF PROJECT (80 characters or less)

The Mechanism of Action of Thyroxine and Its Relation to Cerebral Metabolism

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	L. Sokoloff	Chief, Lab. of Cerebral Metabolism	LCM NIMH
OTHER:	E. E. Kaufman	Research Chemist	LCM NIMH
	T. Nelson	Guest Worker	LCM NIMH

COOPERATING UNITS (if any)
None

LAB/BRANCH
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INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.5	2	1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The goal of this project is the elucidation of the role of the thyroid hormones in the maturation and development of the brain and the influence of the hormones in the regulation of cerebral metabolic processes. Earlier work done previously in this project demonstrated a stimulating action of thyroid hormones on protein biosynthesis. The mechanism of action of thyroxine remains the primary focus of this project. The effects of thyroid hormones on radioactive amino acid incorporation into protein are examined in cell-free protein synthesizing systems from various mammalian tissues, such as liver, brain, reticulocytes, etc. Studies are occasionally carried out in vivo, in which the uptake of amino acids into protein in various tissues is measured in the intact animal or the effects of experimentally induced thyroid disease on various metabolic activities are examined. A wide variety of enzyme purification and assay procedures are also employed. Enzymes are purified from brain or liver homogenates by detergent treatment, salt fractionation, gel adsorption and filtration, ion exchange chromatography, etc. Assays of enzyme activities utilize chemical analyses, spectrophotometric techniques, and Geiger-Mueller and liquid scintillation radioactivity measurements.

Project Description:

This project is directed at the elucidation of the role of the thyroid hormone in the maturation and development of the brain and the influence of the hormone in the regulation of cerebral metabolic processes. A necessary intermediate objective is the determination of the molecular mechanisms of the physiological and biochemical actions of the thyroid hormone. Previous work in this project demonstrated a stimulating action of thyroid hormones on protein biosynthesis. Although the mechanism of this effect on protein synthesis remains a major area of interest, recent work in this project has shifted more to studies of cerebral metabolic processes which may be influenced by the thyroid hormones and may play important roles in a variety of disease states.

The effects of thyroid hormones on various enzyme activities and on radioactive amino acid incorporation into protein are examined in cell-free systems from various mammalian tissues, such as liver and brain, and also in purified enzyme systems. Studies are occasionally carried out in vivo, in which the uptake of amino acids into protein in various tissues is measured in the intact animal or the effects of experimentally induced thyroid disease on various metabolic activities are examined. A wide variety of enzyme purification and assay procedures are employed. Enzymes are purified from brain or liver homogenates by detergent treatment, salt fractionation, gel adsorption and filtration, ion exchange chromatography, etc. Assays of enzyme activities utilize chemical analyses, spectrophotometric techniques, and Geiger-Mueller and liquid scintillation radioactivity measurements.

A major accomplishment of this project thus far has been the demonstration for the first time that thyroxine stimulates protein biosynthesis and that this effect is probably primary to many of the generally recognized physiological actions of the thyroid hormones. Thyroxine, administered in vivo or in vitro, stimulates amino acid incorporation into protein in cell-free rat liver preparations. Conversely, hypothyroidism induced by surgical thyroidectomy, results in a reduction in the rate of amino acid incorporation into protein in similar preparations, indicating that the thyroid effect on protein biosynthesis is tonically active and physiological.

The biochemical mechanism of the thyroxine stimulation of protein biosynthesis has been studied in considerable detail in in vitro systems. These studies have been carried out over a number of years and have demonstrated that the thyroxine stimulation of protein synthesis is exerted by two mechanisms. There is a direct effect on the translational activity of ribosomes leading to accelerated completion and release of newly-synthesized polypeptide chains followed, in vivo only, by an increased synthesis and accumulation of ribosomes, i.e., a transcriptional effect. The transcriptional effect is not a direct action of the hormone but a cellular response or compensation for some earlier effect. The early effect is the one most likely to lead to the elucidation of the primary mechanism of action of the thyroid hormones and is the one which has been of greatest interest to the Section.

The early translational effect on protein synthesis, has, however, also been found to be secondary to an even earlier direct, energy-dependent reaction between thyroxine and the mitochondrial components of the cell. A still unidentified product of this reaction which acts on the ribosomes to stimulate their translational activity has been found. Work has been continuing to isolate, purify, and identify this active factor. It is now known to be a small, dialyzable, heat-stable, acid-labile, anionic, organic, probably non-lipid substance. Continued work on it has increased the knowledge of its properties and narrowed the range of possibilities, but its exact identity remains to be established.

This research has already led to the explanation of the change in sensitivity to thyroid hormones which occurs in brain during development. The brain responds to thyroid hormones in a manner similar to other organs during the period of its development and maturation. Once mature, however, it loses its responsivity to the hormones. This change can be seen in the effects of thyroid hormones on oxidative metabolism, protein synthesis, etc. One outcome of this project has been to show that the loss of sensitivity of the brain to thyroxine with development is the result of some change in the mitochondria so that they lose the ability to react with thyroxine and form the active factor responsible for the stimulation of protein synthesis.

Studies have been continued on the nature of the reaction between thyroid hormones and the mitochondria and the changes produced in mitochondria in the course of the reaction. It has long been known that thyroid hormones cause swelling and structural changes in mitochondria. An interesting finding resulting from the present studies has been the fact that *in vitro* thyroid hormones cause a very rapid loss of pyridine nucleotides, particularly NAD^+, from the mitochondria of liver. Mitochondria of mature brain, which also fail to react with thyroid hormones in the preliminary reaction leading to stimulation of protein synthesis, fail to exhibit this hormonal effect on their NAD^+ content. The effect *in vitro* is so rapid that it precedes the effect on mitochondrial swelling and cannot, therefore, be a consequence of the structural changes in these organelles. That the effect, which was first observed with mitochondria *in vitro*, may have physiological significance is suggested by experiments in which a single injection of a very small dose of hormone *in vivo* leads to a significant reduction in the mitochondrial NAD^+ content. The time course of the effect *in vivo* has been studied; the results indicate that this effect occurs within 8 hours and precedes many of the metabolic effects *in vivo* attributable to thyroid hormones. This effect could have very broad implications. Actions of thyroid hormones are observed on very many metabolic processes in sensitive tissues. NAD^+ is a ubiquitous cofactor for many dehydrogenase reactions which are intimately involved at one point or another in most metabolic pathways. This action of thyroid hormones may, therefore, represent the basis for many subsequent effects of the hormone on a variety of processes.

The thyroid hormones promote the structural, functional, and biochemical maturation of the brain. In addition to the studies of the mechanism of

action of the thyroid hormones, the Section has also been studying the nature of the biochemical changes which occur in the brain with maturation. A prominent developmental change in the brain is a rise in the level of mitochondrial enzymes involved in oxygen consumption and energy metabolism. One of these, D-β-hydroxybutyric dehydrogenase (BDH), was found in this Laboratory to rise with brain development just like other mitochondrial enzymes but, unlike these others, such as cytochrome oxidase and succinoxidase, to decline again gradually following weaning. Evidence has been obtained that the changes in the level of this enzyme in brain are, at least, in part related to nutritional factors, e.g., the high fat content of maternal milk. Hyperthyroidism shifts the developmental pattern in this enzyme to an earlier age (e.g., it causes a more rapid and earlier rise in the enzyme level in the brain, an earlier achievement of its peak level, and an earlier decline). In the course of these studies an interesting observation was made which might be a valuable model for the elucidation of the mechanism of action of thyroxine in mitochondria. Physiologically active thyroid hormones and their analogues were found in vitro to inhibit BDH activity at extremely low concentrations. The inhibition was found to represent a competitive inhibition by the hormone of the binding of the normal substrates, one of which is NAD^+, to the enzyme. The enzyme is membrane-bound in mitochondria. After considerable effort it has been solubilized and partially purified several-fold from both brain and liver. Efforts are continuing to achieve complete purification. The purified enzyme is to be used to study the mechanisms of the thyroxine-inhibition as well as the mechanisms of the regulation of the enzyme's activity through all its changes during postnatal maturation of the brain. It has already been found with the partially purified enzyme that the enzyme requires a phospholipid cofactor normally present in mitochondria for its activity. This cofactor has been identified as a species of lecithin which can be partially substituted for in vitro by commercial beef heart or egg lecithin. The natural cofactor has been partly purified, and progress has been made to identify the nature of its fatty acid composition. The dioleoyl-lecithin is the most active species examined, but the exact identity is still under investigation.

D-β-Hydroxybutyric acid dehydrogenase catalyzes the reversible interconversion between D-β-hydroxybutyrate and acetoacetate, the two ketone bodies found primarily in the liver when fatty acid utilization is increased, as, for example, in diabetes, starvation, high-fat diet, and various states leading to ketosis. Acetoacetate is the primary ketone body formed from catabolism, and it is converted to D-β-hydroxybutyrate by the action of the enzyme. Once formed, however, D-β-hydroxybutyrate cannot be used directly in any of the tissues; it must first be converted back to acetoacetate by the action of the same enzyme. This raises the interesting question of what role the enzyme plays. Why does the body not utilize the directly formed acetoacetate rather than convert it first to D-β-hydroxybutyrate only to oxidize it back to acetoacetate again to utilize it? There is evidence in the literature to suggest that excessive concentrations of acetoacetate in the tissues may be toxic, particularly in brain which has recently been shown to utilize ketone bodies in direct proportion to their levels in the blood. The possibility existed that the role of the enzyme is protective to keep the acetoacetate concentrations low by storing it, in a sense, as D-β-hydroxybutyrate. It is

noteworthy that there are several metabolic conditions characterized by ketosis and coma. Diabetic coma is one example, and recently Reye's Syndrome has been described. This is a frequently fatal disease of childhood which occasionally follows a viral infection, particularly influenza. It is characterized by fatty degeneration of the liver, ketosis, and coma.

Examination of the metabolic pathways and the enzymes responsible for the metabolism of ketone bodies in brain suggests possible mechanisms by which forced excessive use of ketone bodies in brain may lead to depressed cerebral functional activity. The ketone bodies enter the Krebs Cycle after their prior conversion to acetylCoA. The pathway is the conversion of β-hydroxybutyrate to acetoacetate which then displaces succinate from succinylCoA to form acetoacetylCoA. The acetoacetylCoA is then converted to acetylCoA and enters the Krebs Cycle. For every acetoacetate molecule thus metabolized, there is one succinylCoA degraded. SuccinylCoA is required for the formation of GTP which is the precursor of cyclic GMP. Excessive use of ketone bodies can theoretically, therefore, lead to depletion of available GTP and, therefore, also cyclic GMP. There is now a considerable body of evidence that cyclic GMP is the second messenger in the action of the neurotransmitter, acetylcholine. Its depletion can be expected to result in depression of cholinergic functions in brain and lead possibly to depressed functional activity and even coma. The possibility that ketosis may lead to coma by depletion of brain cyclic GMP is currently being studied in this laboratory. A radioimmune assay for cyclic GMP has been established and preliminary results do indeed suggest that in diabetic acidosis there is a depression of cyclic GMP in brain. These studies will be more aggressively pursued during the next year.

Some other recent findings in this project have brought it into closer interrelationship with the project on the [^{14}C]deoxyglucose method. It had been found with the [^{14}C]deoxyglucose method that γ-butyrolactone produces a profound trance-like state with widespread depression of cerebral glucose utilization. The γ-butyrolactone is not the active substance; it is first hydrolyzed to γ-hydroxybutyric acid which is the active agent. γ-Hydroxybutyrate has been identified as a normal constituent of brain, but normally present in very small amounts. Studies are in progress to determine whether or not it is elevated in diabetic coma; preliminary results suggest it is.

At the present time there is very little known about the source and fate of the γ-hydroxybutyrate normally present in brain. There is good evidence that it is oxidized via the Krebs Cycle, but there is no knowledge of its mode of synthesis, and there is nothing at all known of the enzymes involved in its synthesis. Current studies in this laboratory have led to the isolation and purification of an enzyme that catalyzes the interconversion of γ-hydroxybutyrate and its oxidation product, succinic semialdehyde. The enzyme has been completely purified and found to be a monomer with a molecular weight of almost 38,000. The enzyme is a reversible $NADP^+$-linked oxidoreductase which could function both in the oxidation of γ-hydroxybutyrate or its formation by reduction of succinic semialdehyde. Succinic semialdehyde is normally formed in brain from the metabolism of GABA, and this enzyme would provide a link

between γ-hydroxybutyrate and both the GABA metabolic pathway and the Krebs Cycle. The enzyme has been found to be present not only in brain but in even larger amounts in liver. Studies are continuing to determine the role of the enzyme in the origin and fate of brain γ-hydroxybutyrate and the regulation of its level in brain.

The behavioral effects of exogenously administered γ-hydroxybutyrate are very similar to those seen in hibernation, a phenomenon that is still not understood. The possibility that γ-hydroxybutyrate may be involved in hibernation is being examined in this laboratory. Preliminary studies in the hibernating hamster indicate that there is a significant increase in the level of the enzyme that oxidizes γ-hydroxybutyrate in brain but not in liver. These studies will be continued and will be extended to the measurement of γ-hydroxybutyrate levels in brain in hibernation. Progress in this area will be greatly facilitated by the acquisition of a gas chromatographic unit with a nitrogen-detector, which should simplify the assay of γ-hydroxybutyrate.

A unique feature of the cerebral metabolism is its apparent lack of response to high circulating levels of thyroid hormone. An understanding of the basis of this unique behavior may reveal information concerning the metabolism of the brain in health and disease. Also, the mechanism of action of thyroxine has been under investigation for many decades, but thus far a satisfactory explanation of the mechanism by which it increases metabolic rate, stimulates metamorphosis and growth, or causes the many disturbances in body physiology and biochemistry in thyroid disease has eluded investigators. The solution of this problem could then lead to fundamental advances not only in the understanding of how this important hormone regulates bodily functions but also of the nature of bodily processes on which it acts. The clarification of the relationship between its mechanism of action and its effects on development of the brain might also lead to a better understanding of the many biochemical processes underlying the maturation of the brain and the basis of the mental retardation which occurs in neonatal hypothyroidism; e.g., cretinism. Studies in this project on ketone body metabolism may also lead to an explanation of the coma which occurs in a number of metabolic diseases characterized by ketosis.

The project will be continued essentially on the course already indicated. The major avenues to be pursued are: (1) the attempts to isolate, purify, and identify the product of the thyroxine-mitochondrial reaction responsible for the stimulation of protein biosynthesis; (2) further studies on the elucidation of the mechanism of the stimulation of the protein biosynthetic processes; (3) further study of the mechanism of the thyroxine effects on mitochondrial NAD^+ content and its consequences; (4) further studies of the biochemical differences between mature and immature brain and the role of thyroxine in the biochemical changes leading to maturation and development of the brain; (5) purification of brain D-β-hydroxybutyric dehydrogenase; (6) studies of the mechanism of the inhibition of D-β-hydroxybutyric dehydrogenase by thyroxine; (7) studies of the mechanisms of the disturbances in cerebral metabolic processes in coma associated with

metabolic diseases and ketosis; (8) isolation and purification of the enzymes involved in the formation and metabolism of γ-hydroxybutyrate; (9) assay of cyclic GMP and γ-hydroxybutyrate in ketosis-associated coma; and (10) studies of the possible role of γ-hydroxybutyrate in hibernation.

Publications:

1. Sokoloff, L., Fitzgerald, G. G., and Kaufman, E. E.: Cerebral nutrition and energy metabolism. In Wurtman, R.J. and Wurtman, J.J. (Eds.); Nutrition and the Brain, Vol. 1. New York, Raven Press, 1977, pp. 87-139.

2. Sokoloff, L.: Biochemical mechanisms of the action of thryoid hormones: Relationship to their role in brain. In Grave, G.D. (Ed.): Thyroid Hormones and Brain Development. New York, Raven Press, 1977, pp. 73-91.

3. Raskin, N.H., and Sokoloff, L.: Thyroid Disorders. In Goldensohn, E.S. and Appel, S.H. (Eds.): Scientific Approaches to Clinical Neurology, Vol. II. Philadelphia, Lea & Febiger, 1977, pp. 1801-1831.

4. Kaufman, E. E., Nelson, T., Goochee, C., and Sokoloff, L.: Purification and characterization of an $NADP^+$-linked alcohol oxido-reductase which catalyzes the interconversion of γ-hydroxybutyrate and succinic semi-aldehyde. J. Neurochem. (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00882-11 LCM

PERIOD COVERED
October 1, 1977 through September 30, 1978

TITLE OF PROJECT (80 characters or less)
Studies on Regional Cerebral Circulation and Metabolism

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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OTHER:	C. Kennedy	Guest Worker	LCM	NIMH
	M. Shinohara	Visiting Associate	LCM	NIMH
	M. Miyaoka	Visiting Associate	LCM	NIMH
	M. Batipps	Medical Officer (Research)	LCM	NIMH
	C. S. Patlak	Chief, Theoretical Statistics & Math.Br.	B	NIMH
	K. Pettigrew	Mathematical Statistician, Theoretical Statistics & Mathematics Branch	B	NIMH
	H. E. Savaki	Visiting Fellow	LCM	NIMH
	J. McCulloch	Guest Worker	LCM	NIMH
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	J. Magnes	Guest Worker	LCM	NIMH
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LAB/BRANCH
Laboratory of Cerebral Metabolism

SECTION
Section on Developmental Neurochemistry

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH Bethesda, Maryland

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
14.5	9.5	5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A method has been developed for the quantitative determination of the rates of local glucose consumption in the discrete functional and structural components of the brain in conscious or anesthetized laboratory animals. The method is based on the use of [^{14}C]deoxyglucose as a tracer for glucose flux through the hexokinase step. Local [^{14}C]deoxyglucose-6-phosphate concentrations in the tissues of the CNS are measured by a quantitative autoradiographic method. All the theoretical and technical problems have been solved, and the method is now in use for the determination of normal values in the conscious and anesthetized rat. Inasmuch as the autoradiographs of the relative rates of local glucose consumption can be used directly for mapping functionally, and therefore metabolically, linked structures in the CNS, the method is being used to map the functional visual pathways in the monkey. It is also being used to study alterations in the energy metabolism of the discrete functional and structural components of the brain in a variety of physiological, pharmacological, and pathological states.

PHS-6040
(Rev. 10-76)

Project Description:

Previous work in this Laboratory several years ago led to the development of a method for the measurement of the rates of blood flow in the structural and functional units of brain in conscious laboratory animals. The method was based on the uptake of a radioactive, chemically inert gas into the tissues of the brain, and a unique quantitative autoradiographic technique was developed which made possible the measurement by densitometric procedures of the concentrations of the radioactive tracer in the individual structures of the brain down to a resolution of 0.2-0.5 millimeters. The key to the fine resolution of the method was the autoradiographic technique.

Although measurement of local cerebral blood flow is inherently interesting with respect to the physiology, pharmacology, and pathology of the circulatory system, it is of limited value in studies of cerebral functional and biochemical activity. The Laboratory, therefore, addressed itself to the development of a method to measure local cerebral energy metabolism with the same degree of structural resolution because energy metabolism could be expected to relate more closely to local cerebral functional activity. It was always anticipated that the quantitative autoradiographic technique designed for the blood flow method would also be at the heart of such a method. It was necessary, however, to choose an appropriately labeled precursor of cerebral energy metabolism. Oxygen could not be used because there are no suitable radioisotopes of oxygen. [^{14}C]Glucose also appeared to be unsuitable because glucose is too rapidly metabolized, and its radioactive products are too quickly removed from brain. It was, therefore, decided to use [^{14}C]deoxyglucose, an analogue of glucose which is handled qualitatively just like glucose by the transport system in the blood-brain barrier and by the initial enzyme, hexokinase, in the pathway of glucose metabolism. Once phosphorylated, however, the deoxyglucose is trapped, unlike glucose which is metabolized further to carbon dioxide and water. Quantitatively, however, deoxyglucose phosphorylation and glucose phosphorylation or utilization are different inasmuch as the transport carrier and the enzyme discriminate quantitatively between the two substrates. It appeared to be a simple matter to apply the autoradiographic technique to measure deoxyglucose phosphorylation, but to relate it to the steady state rate of glucose flux through the phosphorylation step, which is a measure of the rate of glucose consumption, required the solution of numerous theoretical and technical problems.

A theoretical model, which encompassed all that we knew about deoxyglucose and glucose transport between brain and blood and their metabolism in brain tissue, was constructed, and mathematical relationships to describe the model were developed. Experiments were done on one point or another to evaluate and, if necessary, to revise the model and the mathematical relationships to fit the model closer to the real situation.

It was clear from the model that to determine the rate of glucose consumption from the rate of [^{14}C]deoxyglucose phosphorylation would require the determination of the distribution volumes of deoxyglucose and glucose in the

cerebral tissues and the hexokinase kinetic constants (V_{max} and K_m) for both deoxyglucose and glucose. By appropriate mathematical manipulations, it was possible to segregate all these separate constants into a single "lumped constant" encompassing all of them. It was now necessary to determine only the single lumped constant rather than the six individual ones. Further mathematical analyses revealed how to design an experiment to determine the "lumped constant". Another equation was developed from the model which showed that if the arterial concentration was maintained constant for a sufficient length of time, e.g., at least 20 minutes, then the ratio of the cerebral extractions of deoxyglucose and glucose would reach a constant level equal to the lumped constant. With the help of the Theoretical Statistics and Mathematics Branch, it was found possible to derive from the analyses of plasma disappearance curves of deoxyglucose an intravenous infusion schedule which results in a constant arterial deoxyglucose concentration for up to 45 minutes or longer. Surgical procedures were developed in the rat, monkey, and cat to sample arterial and cerebral venous blood from which the extraction ratios are determined. The lumped constant has been fully determined in the conscious and anesthetized rat; its value is 0.483, and it is unchanged in a variety of physiological and pharmacological states. The lumped constant has recently been determined in the monkey and the cat; the values have been found to be 0.344 and 0.41, respectively.

All the theoretical and technical problems have been solved, and the method has been completely operative. An equation has been derived which relates the rate of glucose consumption to measurable variables and allows the calculation of glucose consumption in the discrete structural and functional units of the brain. The equation prescribes the procedure to be used and the variables to be measured. An intravenous pulse of [^{14}C]deoxyglucose is injected, and arterial plasma concentrations of [^{14}C]deoxyglucose and glucose are measured from the time of injection till 30-45 minutes when the animal is decapitated, and the head frozen. Sections of brain are prepared from which local cerebral tissue [^{14}C]deoxyglucose concentrations are determined by the quantitative autoradiographic technique. From these measured variables and the lumped constant, local cerebral glucose utilization is calculated by the equation. The procedure for calculation has been programmed, and all the calculations are carried out by a computer.

The method has been applied to normal conscious and anesthetized rats and conscious monkeys. The values obtained cannot be directly compared to any other data in the literature since measurement of local cerebral glucose consumption has never previously been possible, but the average of the local values is very close to the reported values for overall average brain glucose utilization in those two species. Anesthesia has been found to depress cerebral glucose consumption throughout the brain. Carbon dioxide inhalation has been shown to depress local glucose consumption even though it increases cerebral blood flow. Local cerebral blood flow has been found in the normal animal to correlate very closely with local cerebral energy metabolism, a relationship which had been hypothesized but never previously proved. Increased local functional activity produced by electrical stimulation or by

experimentally induced focal seizures following local penicillin injection results in markedly increased local glucose consumption in appropriate regions of the CNS. The studies with experimental focal epilepsy have established the existence of a "mirror focus" contralateral to the epileptogenic focus. These results have established once and for all the often debated but never previously proved fact that energy metabolism in the CNS is closely coupled to functional activity as it is in all other tissues.

Studies have been completed in the rat on the effects of γ-butyrolactone, a drug alleged to block specifically dopaminergic synapses in the brain. A surprising result was that this agent, which produces a trance-like state, also depresses local glucose consumption throughout the brain. Indeed, the magnitude of the reduction, which is dose-dependent, can be as great as that seen in profound hypothermia, which is used by cardiac and neurosurgeons to reduce energy metabolism in the brain and thus protect it during surgical procedures necessitating interruption of cerebral blood flow. The effects of the drug are completely reversible. Normal consciousness, EEG, and behavior are recovered approximately 4 hours after the injection, and the animal appears normal. This finding may have enormous practical potentialities. It is conceivable that the beneficial effects of hypothermia without its potentially dangerous side-effects and complex technical procedures may be achieved with this drug. These studies will be continued to ascertain whether, in fact, it is safe to try it out in man for surgical procedures.

Studies have been completed on the effects of LSD and of acute morphine administration, morphine addiction, and morphine withdrawal on local cerebral glucose utilization. The morphine studies are being carried out in collaboration with Dr. Werner Klee. These studies have demonstrated selective, dose-dependent depressions of energy metabolism in specific regions of the brain. Morphine addiction has been found to result in significant reductions of glucose utilization in a number of structures of the brain, particularly those of the limbic system. Acute withdrawal from addiction by naloxone administration results in a reversal of this reduction in several of those structures, most prominently in the habenula. An interesting observation is that naloxone alone, when administered to normal rats, reduces glucose utilization in many of the same structures affected by morphine addiction. Naloxone, which is used as a morphine antagonist, has generally been believed to be inactive by itself. These results suggest, however, that it is active, possibly by acting on the receptor sites normally acted upon by the recently discovered endogenous "opiate polypeptides", i.e., the enkephalins.

Studies have been completed on the effects of amphetamine in the rat. This dopaminergic agonist produces pronounced increases in glucose utilization in selected structures of the brain, in particular the components of the extra-pyramidal dopaminergic motor system, i.e., caudate nucleus zona compacta and zona reticulata of the substantia nigra, etc. A markedly affected structure was the sub-thalamic nucleus, which had previously not been suspected to be dopaminergic, but in follow-up studies based on those of this laboratory, has been found to contain an active dopamine-sensitive adenylcyclase. Studies are

currently in progress in collaboration with Drs. Agu Pert and William Bunney of the Adult Psychiatry Branch, NIMH, on the supersensitization of dopaminergic synapses by chronic administration of dopamine-blocking drugs.

Studies on the effects of the β-adrenergic blocking agent, propranolol and the α-adrenergic blocking drug, phentolamine, have led to surprising results. These drugs have directly opposite effects selectively on the auditory system of the rat. Propranolol, which is presently being widely touted as an anti-hallucinogenic agent in schizophrenia and other psychoses, was found to depress markedly the rates of glucose utilization throughout the entire auditory system from the cochlear nucleus to the auditory cortex. In contrast, phentolamine, which has mild depressant effects in most other parts of the brain, has exactly the opposite effects in the components of the auditory system, namely a pronounced increase in glucose utilization. Studies are currently in progress extending the investigations to other adrenergic agonistic and antagonistic drugs.

In the course of the development of the method it became apparent that even without complete quantification, the autoradiographs represented pictorial displays of the relative rates of energy consumption in all parts of the brain. For example, they demonstrated clearly that the synapse-rich areas, e.g., layer 4 of the neuropile in the cerebral cortex, are the most metabolically active areas of the cortex. It also became apparent that inasmuch as local glucose consumption changes in response to changes in functional activity, regions of the brain which were turned on or off functionally and metabolically in altered physiological states could be mapped simultaneously throughout the brain. The efficacy of this technique of mapping by "evoked metabolic response" has been demonstrated in the visual and auditory systems of the rat and monkey. All the regions involved in visual functions have, for example, been shown to alter activity and be displayed on the autoradiographs after occlusion of vision of one eye and stimulation of the other. By this technique the visual cortical locus of the representation of the blind spots of the visual fields has been demonstrated in the monkey and its shape and dimensions defined. The resolution of the method is surprisingly good. It has clearly demonstrated the ocular dominance columns described by Hubel and Wiesel, which are believed to represent the structural and functional organization of the visual cortex responsible for the integration of the two images from the two eyes in animals with binocular vision. Application of the method to the newborn monkey has demonstrated that these columns are already organized at birth. Patching of one eye in the newborn for 3 months leads to a reorganization of the visual cortex so that the portions representing the blocked eye are taken over by the portions of the visual cortex subserving the open eye. Such plasticity is not present in the mature monkey brain.

Studies are in progress to determine the mechanisms by which glucose utilization is coupled to functional activity. One possibility is that increased functional activity increases extracellular K^+, and it is the increased extracellular K^+ that stimulates energy metabolism by activation of

the Na^+,K^+-ATPase. Experiments have been carried out in which KCl is applied to the parietal cortex trans-durally by its topical application through a hole in the skull or directly on the pial surface of the cortex. Such application of the KCl markedly stimulates glucose consumption in the underlying cortex, probably by direct action, while depressing metabolic activity in subcortical structures, probably by reflex or neurogenic reduction of functional activity. This treatment is known to cause the phenomenon of spreading cortical depression during which the DC potential of the cortex is shifted negatively. Studies during the recovery from spreading depression (i.e., immediately following the return of the DC potential to normal) have demonstrated that there is still increased cortical glucose utilization, but it is distributed in a columnar pattern traversing the thickness of the cortex. These results give additional support to the accumulating evidence of the columnar organization of functional activity in the cerebral cortex.

The method has also been used to study the acute and delayed effects of hyper-osmolar opening of the blood-brain barrier. The barrier was opened by unilateral carotid arterial injections of hyper-osmolar mannitol. Shortly after the barrier is opened, there is metabolic evidence of punctate regions of seizure-like activity on the side of the barrier opening. The effect is apparently reversible, for those areas of markedly increased metabolic activity return to normal after a period of an hour or more following opening of the barrier.

Recent technological developments with computerized axial tomography have made it possible to scan the human brain for local tissue concentrations of positron-emitting isotopes. Experiments have, therefore, been carried out with [^{18}F]fluoro-deoxyglucose, a positron-emitting analogue of [^{14}C]deoxyglucose, and the results demonstrate that it can be used in place of [^{14}C]-deoxyglucose as the tracer for the deoxyglucose method. This now makes it possible to use the method in man. In collaboration with Dr. M. Reivich of the U. of Pa. and a number of other investigators, the method has been adapted for human use. The NINCDS has ordered a scanner which should be operational by the end of the calendar year. This instrument will be available for our use, and we hope to apply it to studies of psychotic states in man.

The [^{14}C]deoxyglucose method measures glucose utilization which correlates with and appears to be a marker for local cerebral functional activity. There are, however, other parameters of cerebral function and structure of interest. We have, therefore, recently initiated studies on a comparable method for the quantitative determination of local cerebral protein synthesis. It is expected that this method should allow more pertinent and probing studies of the development of brain structure and function in normal and abnormal states. The model is similar to that of the glucose method, and progress is being made to bring the method to an operational state.

Several lines of further investigation are planned in this project. One line is methodological by which it is hoped to improve the resolution of the method to the microscopic or single cell level. Another line is to exploit the [^{14}C]deoxyglucose method at its present state to studies of the effects of

aging, psychoactive drugs, metabolic comas, development and maturation of the brain, and hormones such as thyroxine known to have important actions on the maturation of the brain. Finally, the local protein synthesis method should be soon developed to the operational level and be ready for use in studies of brain development.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00886-01 LCM

PERIOD COVERED

October 1, 1977 through September 30, 1978

TITLE OF PROJECT (80 characters or less)

Circadian Rhythmicity of Glucose Utilization in the Rat Suprachiasmatic Nucleus

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	W. J. Schwartz	Medical Officer (Research)	LCM	NIMH
	C. B. Smith	Staff Fellow	LCM	NIMH

COOPERATING UNITS (if any)

Laboratory of Neurophysiology, NIMH

LAB/BRANCH

Laboratory of Cerebral Metabolism

SECTION

Section on Developmental Neurochemistry

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.5	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In this study the autoradiographic 2-deoxy-D[^{14}C]glucose method of determining glucose utilization was applied in an attempt to demonstrate a circadian rhythm of an intrinsic property of the rat suprachiasmatic nucleus (SCN). It was found that (1) SCN glucose utilization could be entrained to a 24 hr environmental light-dark cycle and phase shifted 180° by reversing the external entraining cycle. (2) This metabolic rhythm appeared to persist in the absence of any external light-dark cues but it was arrhythmic after 10 days of constant light. (3) The effect of ambient darkness during the light phase of the entraining cycle appeared to "unmask" an underlying endogenous metabolic activity of the nucleus. (4) When animals maintained in constant darkness or constant light were exposed to either ambient light or darkness, respectively, the resulting pattern of altered SCN glucose utilization suggested that the sensitivity of the nucleus to ambient light during constant conditions may have been a function of the phase of an endogenous SCN oscillation.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives. The suprachiasmatic nucleus (SCN) in the anterior hypothalamus has been proposed as the endogenous circadian oscillator in mammals. The evidence is based on the fact that a retino-hypothalamic pathway terminating in the SCN has been identified and appears to be necessary for the entrainment of circadian rhythms to the environmental light-dark cycle and that lesions of the SCN abolish a wide spectrum of overt circadian rhythms. In order to demonstrate that a directly measurable property of the SCN itself in intact (unlesioned) animals is affected by environmental light and exhibits circadian rhythmicity, the autoradiographic 2-deoxy-D-[^{14}C]glucose method was used to determine glucose utilization of rat SCN under various lighting conditions.

Methods. A total of 88 adult, male Sprague-Dawley albino rats, weighing between 300 and 400 grams each, were used in experiments conducted from October, 1977, to April, 1978. Rats were housed in individual activity-wheel cages in a room with controlled artificial lighting. During "light" phases, the cages were bathed in approximately 30 ft-c illumination from overhead fluorescent bulbs; during "dark" phases, less than 0.01 ft-c illumination was provided by two Kodak 15 watt safelights with red (1A) filters. Individual running-wheel activities were recorded.

Groups of rats were subjected to various lighting regimens before the determination of SCN glucose utilization. At least two hours after catheterization of the femoral vein and artery, animals were injected intravenously with 2-deoxy-D-[^{14}C]glucose while they were alert and restrained. After 45 min the animals were killed by an intravenous injection of sodium pentobarbital; and their brains were removed, frozen in 2-methylbutane (-40°C), and serial coronal sections 20 μm thick were cut through the region of the SCN. Sections were dried and autoradiographs prepared.

After autoradiography, the sections were stained with cresyl violet to determine the location and extent of the SCN. Optical densities of the SCN and other selected brain regions were read on a Photoscan System P-1000 densitometer (Optronics International, Inc.). Optical densities of the left and right SCN were recorded for each section along the 600 μm length of the nucleus, and mean glucose utilization of the entire nucleus was calculated.

Major Findings. The results of these experiments indicate that the rat SCN has properties consistent with a role as an endogenous circadian oscillator. The glucose utilization of the SCN can be entrained to a 24-hr environmental light-dark cycle (LD) and phase-shifted 180° by reversing the external entraining cycle (DL). Glucose utilization is high during the light phase and low during the dark phase. This metabolic rhythm appears to persist in a somewhat modified form in the absence of any external light-dark cues, i.e., during constant darkness or after bilateral enucleation. After 10 days in these constant conditions, the nucleus is metabolically active for a shorter time and at a lower level of glucose utilization during the subjective day than it had been during the light phase of either an LD or DL cycle. However, after 10 days of constant light, SCN glucose utilization appears

arrhythmic and at an intermediate level. When animals in the "D" phase of an LD cycle are exposed to light, SCN glucose utilization increases from low levels to high values comparable to those observed during the "L" phase. On the other hand, dark exposure during the "L" phase results in a pattern of altered SCN glucose utilization similar to that observed during the subjective day in constant darkness or after bilateral enucleation. Dark exposure thus appears to "unmask" the endogenous activity of the nucleus. When animals in constant darkness or constant light are exposed to either light or darkness, respectively, the resulting pattern of altered SCN glucose utilization suggests that the sensitivity of the nucleus to ambient light during constant conditions may be a function of the phase of an endogenous SCN oscillation.

Proposed Course. The experimental work on this project has been terminated. Preliminary results were reported at the Ninth Annual Meeting of the American Society for Neurochemistry [Trans. Am. Soc. Neurochem. 9, 96 (1978)]. In addition, the work will be presented at the Naito International Symposium on "Biorhythm and Its Central Mechanism" in August, 1978. A manuscript of this work has been prepared and will be submitted to Nature as an article.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00887-01 LCM

PERIOD COVERED
October 1, 1977 through September 30, 1978

TITLE OF PROJECT (80 characters or less)
The Extended Visual System of the Macaque Monkey

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	C. Kennedy	Guest Worker	LCM	NIMH
	M. Mishkin	Research Physiologist	LN	NIMH
OTHER:	C.D. Jarvis	Research Psychologist	LN	NIMH
	M. Shinohara	Visiting Associate	LCM	NIMH
	M. Miyaoka	Visiting Associate	LCM	NIMH

COOPERATING UNITS (if any)
Laboratory of Neuropsychology, NIMH (Proj. No. Z01 MH 02033-01 LN)

LAB/BRANCH
Laboratory of Cerebral Metabolism

SECTION
Section on Developmental Neurochemistry

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.50	1.25	0.25

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

It has been shown in years of experimental work that the visual system of the primate is far more extensive than was long thought to be the case. Studies employing the effects of lesions, the responses of single cells, and anatomic methods have shown that a cortical visual pathway extends forward, well beyond the prestriate region, in the inferior portion of the temporal lobe. This extensive cortical system appears to process visual stimuli with serial increments in complexity as impulses cascade forward from the striate cortex. Some evidence has accrued that impulses then enter the limbic system via the amygdala and that in this part of the brain the visual stimuli receive motivational value. In view of the previously demonstrated ability of the 2-deoxyglucose method to map functional visual pathways(e.g., to demonstrate the ocular dominance columns, the orientation columns, the cortical locus of the blind spot of the retina) it was thought that it might be possible to map this extended visual system in detail and therefore the present collaboration was

undertaken. In a series of experiments in which one hemisphere of a monkey's brain was deprived of visual stimuli by surgical section of one optic tract and the forebrain commissures it was indeed possible to demonstrate in exquisite detail the extent of this large region of visually responsive cortex. The results were reported at the recent meetings of the American Academy of Neurology (abstract, 1978). In the presentation, color coding of autoradiographs permitted the depiction of differences in rates of local glucose utilization in all areas of a coronal section simultaneously. This provided a unique amalgamation of anatomic, physiologic and biochemical information in a single picture. It marked the limits of the visual cortical analyzer in detail (notably within brain sulci) and also marked zones within the limbic system which can be activated by visual input.

Plans are underway to study other sensory systems (auditory and somatosensory) in a similar manner to test the hypothesis that other portions of the temporal lobe may be similarly involved in processing these modalities.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00888-01 LCM

PERIOD COVERED

October 1, 1977 through September 30, 1978

TITLE OF PROJECT (80 characters or less)

Maturation of Cortical Function in the Macaque Monkey

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	C. Kennedy	Guest Worker	LCM	NIMH
	P. Goldman	Research Physiologist	LN	NIMH

COOPERATING UNITS (if any)

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LAB/BRANCH

Laboratory of Cerebral Metabolism

SECTION

Section on Developmental Neurochemistry

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.50	0.25	0.25

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☐ (a1) ☐ (a2) INTERVIEWS

KEYWORDS (200 words or less - underline keywords)

In 1975 the Laboratory of Cerebral Metabolism had extended its earlier developmental studies (local cerebral blood flow) to those of local cerebral metabolism in which the 2-deoxyglucose method was applied. Striking differences were noted both in distribution and rate of glucose utilization between newborn and pre-pubescent monkeys. These findings suggested that the 2-deoxyglucose method would be useful in mapping the pathways involved in behavioral development. This collaboration was therefore undertaken with Dr. Goldman because of her many studies in the area in which other methodology has been employed. To date two experiments have been carried out in which learning ability will be examined. The results are not known.

PHS-6040
(Rev. 10-

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00900-22 LCM

PERIOD COVERED

October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Biochemical Studies on Myelin and Myelin Basic Protein

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. E. Martenson	Research Chemist (Biochem.)	LCM	NIMH
Other:	G. E. Deibler	Chemist	LCM	NIMH
	M. W. Kies	Chief, Section on Myelin Chemistry	LCM	NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Cerebral Metabolism

SECTION

Section on Myelin Chemistry

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	1.5	1.0

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A study of the conformational flexibility of the myelin basic protein has shown that the molecule is not stabilized to any significant extent by intramolecular interactions. As the net charge on the molecule increases from nearly zero at pH 11 to +41 at pH 2-3, the molecule gradually expands with no evidence of a cooperative transition. At pH 2 or pH 7 the molecule continually contracts as the ionic strength increases. Relatively low concentrations of guanidine hydrochloride are sufficient to cause an expansion of the protein with no evidence of a cooperative transition. There appears to be very little structure in the molecule which is stable to changes in the external milieu. This conformational flexibility of the protein may be necessary for maximal interaction of the protein with lipids in the myelin sheath.

PHS-6040
Rev. 10-76)

Project Description:

The objectives of this study are to continue to examine the relationships between chemical structure and biological activities of the myelin basic protein. The biological activities include the ability to (A) induce delayed hypersensitivity leading to an autoimmune disease, experimental allergic encephalomyelitis (EAE), (B) induce delayed hypersensitivity without necessarily causing EAE, (C) induce the formation of circulating anti-basic protein antibodies without concomitant induction of EAE, and (D) provide structural stability in myelin by its interaction with lipids in the membrane.

The biochemical methods used include (1) polyacrylamide gel electrophoresis; (2) amino acid analysis; (3) chromatographic fractionation of proteins and peptides; (4) paper chromatography; (5) high voltage electrophoresis; (6) thin-layer chromatography; (7) specific chemical and enzymic cleavage of myelin basic proteins; and (8) analytical gel filtration.

Our earlier studies on the region of the guinea pig myelin basic protein that is highly encephalitogenic in Lewis rats have shown that full activity is present in a proteolytic fragment, residues 61-88. Shorter fragments (residues 72-88 and 72-84) are also highly active, but less so than the longer fragment. In order to evaluate the importance of the peptide chain immediately preceding residue 72 in augmenting EAE in Lewis rats, we are currently examining synthetic peptides. These peptides have been synthesized by Dr. Janis Young at the Space Sciences Laboratory, University of California, Berkeley (supported by a grant from The Kroc Foundation to Dr. M. W. Kies). To date the following peptides have been synthesized: peptides 72-84, 71-84, 70-84, 69-84, and 68-84. These synthetic peptides are quite heterogeneous and are being purified in our laboratory prior to bioassay. We hope to be able to demonstrate enhanced encephalitogenic activity with increasing chain length.

During the past year I have completed studies on the conformational flexibility of the myelin basic protein. The hydrodynamic behavior of the protein has been studied by gel filtration through Sephadex G-100 under conditions which have included variations in pH from 2 to 12, variations in ionic strength from 0.01 to 1.5 M at pH 2 and from 0.1 to 2 M at pH 7, and variations in guanidine hydrochloride concentrations from 0 to 6 M. A number of well characterized compact globular proteins have been subjected to the same conditions for comparison and validation of the procedure. The results demonstrated the lack of significant intramolecular stabilization in the basic protein.

By the use of published values of the hydrodynamic volume and the diffusion coefficient of the basic protein at pH ~5, together with the R_e values determined by gel filtration, it has been possible to describe the expansion and contraction of the protein in terms of changes in hydration or dimensions, or both, of an equivalent prolate ellipsoid of revolution.

In terms of a prolate ellipsoid model in which hydration is taken into account, the asymmetry of the molecule is only half as great as that which has been commonly accepted. The lower asymmetry and the marked flexibility of the molecule are not compatible with the popular model of the protein as a double chain, extended structure resulting from a hypothetical hairpin turn at the triproline sequence (residues 98-100).

The asymmetry, open conformation, and flexibility of the basic protein molecule provide a relatively large number of hydrophobic contact surfaces capable of interactions leading to aggregation once the net charge on the molecules is reduced sufficiently or the surface tension of the solvent is increased sufficiently by the addition of an appropriate neutral salt. The extensive exposure of nonpolar, as well as polar and charged, residues to the external milieu undoubtedly plays a key role in the ability of the protein to penetrate or deform lipid monolayers; increase the permeability, alter the thermotrophic properties, and promote the fusion and aggregation of liposomes; and according to current views, maintain the structure of myelin by holding the cytoplasmic surfaces of the oligodendroglial plasma membrane in apposition.

Myelin basic protein is under intensive investigation in this and a number of other laboratories to determine its role in the structural integrity of the myelin sheath, where it accounts for 30% of the myelin proteins. One of the major problems concerning the protein is its conformation and the nature of the other myelin constituents with which it interacts in vivo. Intimately related to the structure of the protein is its involvement in an experimental autoimmune disease of the CNS, experimental allergic encephalomyelitis (EAE), where it serves as a potent auto-antigen capable of specifically sensitizing lymphocytes which initiate a delayed-type hypersensitivity reaction in the CNS tissues leading to paralytic lesions. To study the properties of the protein responsible for the induction of the delayed hypersensitivity, both with and without concomitant production of EAE, and those responsible for induction of antibody formation alone, we are examining various areas of the basic protein for the relevant activities. Through these studies, as well as physicochemical studies of the protein, we hope to obtain information relevant to the detailed structure of the protein and to molecular events which occur in the afferent part of immune responses in general, and possibly during the early phases of autoimmunity in humans, specifically in multiple sclerosis.

Publications:

Morris, R. W., Cagan, R. H., Martenson, R. E., and Deibler, G.: Methylation of the lysine residues of Monellin. Proc. Soc. Exp. Biol. Med. 157: 194-199, 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00901-23 LCM

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)
Immunologic Reactivity of Myelin Basic Protein

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. W. Kies	Chief, Section on Myelin Chemistry	LCM	NIMH
Other:	H. D. Webster	Head, Section on Cellular Neuropathology	LNNS	NINCDS
	N. Sternberger	Research Associate		

COOPERATING UNITS (if any)
Laboratory of Neuropathology and Neuroanatomical Sciences, NINCDS

LAB/BRANCH
Laboratory of Cerebral Metabolism

SECTION
Section on Myelin Chemistry

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Specific antisera to myelin basic protein (BP) and its fragments have been used to study (A) the relationship of antibody-binding to conformation; (B) location of specific antigenic and encephalitogenic regions within the BP molecule; and (C) cytochemical localization of BP in developmental studies.

Rabbit antisera to mammalian central nervous system (CNS) BP have been analyzed for their binding specificity to ^{125}I-labelled fragments of BP. From our data, we conclude that rabbit lymphocytes recognize and produce antibody to an amino acid sequence in the N-terminal region between residues 1 and 36; at least two peptide sequences in the next 52 residues (37-88); and one or more sequences in the last 80 residues (89-169). Individual animals may recognize different combinations of regions but no one site stands out as being a major binding site as was the case with guinea pig anti-BP sera.

Project Description:

(A) Information on the conformation of basic protein (BP) in solution has been gained from binding studies involving BP and its fragments with anti-BP (or fragment) antibody induced in rabbits. Although other investigators have reported the existence of a "buried" region in the BP molecule between residues 37 and 88 which failed to react with antibody, our antibody binding data indicate otherwise:

1) The existence of a "buried site" is impossible if BP exists as a flexible polyelectrolyte without any significant intramolecular interactions, as was suggested by Dr. Martenson's physical chemical data.

2) Furthermore, if the site had been truly "buried" BP immunization would not have induced antibody to that region (i.e., helper T-cells and B-cells would not have "recognized" the site).

3) Our antigen-antibody binding studies also provide abundant evidence that no region of the molecule is prevented from binding antibody. Whether immunized with whole BP or isolated fragment, some rabbits produce antibodies that react equally well with a given sequence whether the protein itself or its proteolytic fragment is used to study binding capability. It is possible that some peptide fragments may be capable of conformations different from the conformation of the peptide sequence in the protein itself and therefore may occasionally induce antibodies which do not cross react well with the identical sequence in the whole protein.

(B) With the purified fragments which we used for rabbit antibody studies we have reinvestigated the location and number of rabbit encephalitogenic sites. The identity and limiting sequences of guinea pig and rat encephalitogenic sites have been well-established but comparable studies have not been done on rabbits. Early reports by other investigators suggested that fragment 43-88 isolated from whole tissue contained the active site for rabbits. Our work established the fact that the active peptide had been derived from BP during the extraction process and did not exist as a separate encephalitogen in situ. However, at the time we did not question the validity of the identification of the encephalitogenic fragment as residues 43-88.

Our recent bioassays of purified and carefully identified fragments have cast some doubt on the earlier report. We have found that 6 of 12 rabbits injected with 50-250 mcg of bovine residues 1-42 have succumbed to experimental allergic encephalomyelitis (EAE) while none of 8 rabbits injected with 10-250 mcg of bovine residues 43-88 have developed any signs of EAE.

In repeat experiments with fragments isolated from guinea pig BP we found two of 2 rabbits developed EAE after injections of 200 mcg of fragment 1-36; none of 2 after 200 mcg of fragment 37-88, and one of 2 developed clinical signs of EAE after 200 mcg of fragment 43-88. We are repeating the

latter assays. It is possible that there are 2 sites on the molecule (one each in residues 1-42 and residues 43-88) but it is also possible that fragment 43-88 in the original report was not correctly identified.

(C) The collaborative studies with Drs. Sternberger, Itoyama and Webster (NINCDS) have shown that BP can be demonstrated immunocytochemically in oligodendroglia of the developing rat CNS before it appears in newly formed myelin. This immunocytochemical technique will have important applications in studies of pathologic conditions, both those induced experimentally and those occurring in human diseases (e.g., multiple sclerosis).

Publications:

Eng, L. F., Lee, Y-L., Williams, K., Fukayama, G., Gerstl, B., and Kies, M. W.: Clearance of myelin basic protein from blood of normal and EAE rabbits. In Palo, J. (Ed.): Myelination and Demyelination. New York, Plenum Publishing Co., 1978, pp. 329-346.

Kies, M. W.: Autoimmunity in multiple sclerosis: do we have an experimental model? In Palo, J. (Ed.): Myelination and Demyelination. New York, Plenum Publishing Co., 1978, pp. 277-288.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00902-13 LCM

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)
Induction and Prevention of Experimental Allergic Encephalomyelitis (EAE)

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	B. F. Driscoll	Guest Worker	LCM	NIMH
Other:	M. W. Kies	Chief, Section on Myelin Chemistry	LCM	NIMH
	J. R. Richert	Guest Worker	LCM	NIMH

COOPERATING UNITS (if any)
National Multiple Sclerosis Society
E. C. Alvord, Jr., University of Washington School of Medicine, Seattle, Wash.

LAB/BRANCH
Laboratory of Cerebral Metabolism

SECTION
Section on Myelin Chemistry

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	1.5	1.0

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

An attempt has been made to analyze immunologic mechanisms in EAE by in vitro examination of cells from BP-sensitized animals. It is well known that sensitized cells are stimulated to divide (proliferate) when they are exposed to specific antigen in vitro. Thus, cells from animals sensitized with BP in complete Freund's adjuvant (CFA) undergo proliferation in the presence of either BP or PPD. (CFA contains tubercle bacilli which sensitize the animal to PPD.)

Our data on the stimulation of sensitized cells with specific antigen in vitro indicate that: 1) proliferation in culture in the presence of BP correlates well with the disease state, and 2) this proliferation is not induced primarily by the site on the molecule responsible for disease induction. The latter observation was somewhat surprising and led us to investigate other effects of antigen in vitro.

PHS-6040
(Rev. 10-76)

Project Description:

Experimental animals sensitized with either myelin basic protein (BP) or whole central nervous system (CNS) tissue develop experimental allergic encephalomyelitis (EAE). This disease is known to be caused by an immune response to BP, mediated by a class of lymphocytes referred to as thymus-dependent lymphocytes (T cells). There are several in vitro assays which can be used to test for the presence of antigen specific lymphoid cells from sensitized animals. One of these tests is the lymphocyte proliferation assay. Cells from an animal sensitized with antigen proliferate when they are exposed to the same antigen in culture. In the presence of antigen, incorporation of ^{3}H-thymidine is enhanced. Although the proliferation assay has been widely used, the precise role, if any, of the proliferating cells in the cell mediated immune (CMI) response is still open to question.

In the case of EAE, this question can be answered somewhat more directly than in other immune systems. The quantitative relationships between sensitization with BP and disease severity have been carefully worked out. The BP molecule has been sequenced and the site responsible for disease induction in guinea pigs is known. Furthermore the minimal sequence required for disease induction has been determined. A synthetic nonapeptide corresponding to residues 113-121 induces definite clinical and histologic disease although the reaction is somewhat milder than that induced by the whole protein. Loss of even one residue from the nonapeptide results in complete loss of reactivity. In addition to this synthetic peptide we have available many other well-characterized fragments of the molecule, some of which do not contain the encephalitogenic sequence.

Before determining whether the various fragments could replace the whole molecule as the "stimulating" antigen, it was necessary to establish the correlation between in vitro stimulation and presence of disease in vivo.

BP-sensitized cells from Strain 13 guinea pigs (susceptible to EAE) are stimulated by BP in culture. On the other hand, BP-sensitized cells from Strain 2 guinea pigs (not susceptible to EAE) are only slightly stimulated in culture. Likewise, cells from Strain 13 guinea pigs protected against induction of EAE by prior injections of BP in incomplete Freund's adjuvant are not stimulated in culture. Thus, the presence of the disease state (and CMI to BP) can be readily detected by cell proliferation in culture and cells from animals that do not develop disease after sensitization with BP in complete Freund's adjuvant (CFA) do not proliferate in culture. However, this does not prove that the cells that proliferate are actively involved in the disease process.

To settle this uncertainty we tested the cells from BP-sensitized animals with various fragments of the BP molecule. The data indicate: 1) many regions of the molecule that do not contain the encephalitogenic site cause stimulation of cells in culture; 2) the synthetic encephalitogenic nonapeptide and other small peptides (~20 amino acids) that include the encephalitogenic site do not stimulate cells in culture; and 3) by deduction, the

stimulation obtained with larger peptides (~80 amino acids) that contain the encephalitogenic site, probably was not caused by the site itself but by other regions of the peptide.

We conclude that this test correlates well with CMI to BP (and therefore disease) but that regions of the BP molecule other than the encephalitogenic site are responsible for this stimulation. These data would indicate, therefore, that the cells proliferating in culture are probably not the cells responsible for disease.

These results have great significance to many of the current studies on the role of autoimmunity in multiple sclerosis. If indeed the cells that proliferate in culture are not the cells which initiate damage in the CNS the studies carried out in patients on leukocyte proliferation in the presence of BP are probably of no significance.

Studies are now underway to determine the function of these proliferating cells in vivo and to separate them from the cells responsible for disease.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00903-01 LCM

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)
Preparation and Identification of Peptides Derived From Myelin Basic Protein

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	G. E. Deibler	Chemist	LCM	NIMH
Other:	M. W. Kies	Chief, Section on Myelin Chemistry	LCM	NIMH

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Cerebral Metabolism

SECTION
Section on Myelin Chemistry

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Myelin basic protein (BP) presents a unique opportunity for a detailed study of the relationship between structure and biological activity--it has been completely sequenced and there is an impressive backlog of information on its encephalitogenic activity in several species; its ability to induce and bind antibody and its ability to bind other biochemical compounds or structures, specifically membrane constituents.

In order to study the relationship between structure and activity, we have prepared several well-characterized fragments of the protein by controlled proteolytic digestion and have purified several synthetic peptides prepared for us by Dr. Janis Young, Space Sciences Laboratory, University of California, Berkeley. Our purpose is to test the ability of these peptides to replicate the biological activity of the protein. By using fragments from different regions of the protein one can "map" active sites and define the minimal structural requirements for activity.

Project Description:

Last year Dr. Nomura isolated several small peptides from basic protein (BP) by treatment of the protein or a peptic fragment of BP, residues 89-169, with Staphylococcus aureus V8 protease.

This enzyme was reported to cleave at glutamyl residues in ammonium ion buffers and at both glutamyl and aspartyl residues in phosphate buffer. Our analytical studies of these peptides have demonstrated that BP is cleaved by V8 protease in the following manner: The first cleavage occurs at Asp(32)-Thr(33); the second at Asp(37)-Ser(38); the third at Glu(118)-Gly(119); and finally, Gly(127)-Gly(128) is cleaved. Not only was the Asp bond in BP more susceptible to V8 protease action than it was in other proteins but the Gly-Gly bond (127-128) was also split.

Identification of these fragments required development of special techniques for C-terminal analysis, since the usual carboxypeptidase A and B preparations did not attack C-terminal Glu or Asp residues. Carboxypeptidase Y, which has much broader specificity than A and B, has not been entirely satisfactory because of its high endopeptidase activity. The problem of good C-terminal analysis is still being studied.

The synthetic peptides available for our studies were synthesized by Dr. Young at the Space Sciences Laboratory, University of California, Berkeley (supported by a grant from the Kroc Foundation to Dr. M. W. Kies). They were partially purified in our laboratory by cycling through Sephadex columns. Synthetic peptides present a special problem in purification because the physical and chemical differences between the major products and the impurities are small. Because the impurities are closely related peptides lacking one or more of the amino acid residues, they do not interfere with tests for biological activity provided they represent a relatively small part of the sample.

With purified peptides estimated to contain at least 10% of these imperfect sequences we tested the encephalitogenic activity in guinea pigs of a family of peptides containing the nonapeptide (residues 113-121) to ascertain the effect of additional residues on encephalitogenicity.

The nonapeptide itself has weak activity compared to the whole protein. The addition of two amino acids on the N-terminal end and seven on the C-terminal end enhanced the encephalitogenic activity. (This peptide corresponds to residues 111-128.) The two extra N-terminal amino acids appear to be more critical to activity than the seven C-terminal ones inasmuch as deletion of the two N-terminal residues, giving a fragment corresponding to residues 113-128, resulted in a reduction of activity essentially to the level of that shown by the nonapeptide. Further studies are in progress to ascertain the effect of size and conformation on the biological activity of different fragments of the BP molecule.

Publications:

Deibler, G. E., Driscoll, B. F., and Kies, M. W.: Immunochemical and biochemical studies demonstrating the identity of a bovine spinal cord protein (SCP) and a basic protein of bovine peripheral myelin (BF). J. Neurochem. 30: 401-412, 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00931-05 LGCB

PERIOD COVERED

October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Studies on the Characteristics and Regulation of S-Adenosylhomocysteine Hydrolase

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	G. L. Cantoni	Chief, Lab. Gen. Comp. Biochem.	LGCB NIMH
	P. K. Chiang	Sr. Staff Fellow	LGCB NIMH

COOPERATING UNITS (if any)

J. P. Bader, Lab. of Tumor Virus Genetics, NCI
E. J. Leonard, Lab. of Immunobiology, NCI

LAB/BRANCH

Laboratory of General and Comparative Biochemistry

SECTION

Section on Proteins

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1-5/6	5/6	1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Adenosylhomocysteine hydrolase (E.C. 3.3.1.1) from calf liver has been purified to homogeneity by crystallization. It has a molecular weight of 237,500, and can be separated into two isoelectric focusing variants. When electrophoresed with sodium dodecyl sulfate, a major subunit of a molecular weight of 60,000 was found; five other minor subunit variants were also observed, with molecular weights ranging between 50,000 and 57,000. The presence of enzyme-bound NAD was confirmed, probably one NAD molecule bound/enzyme subunit. In addition to adenosine, 3-deazaadenosine was found to be an effective substrate as well in the direction of synthesis; but in the direction of hydrolysis, 3-deazaadenosine becomes a potent inhibitor. When tested in cell systems both 3-deazaadenosine and 5'-deoxy-5'-(isobutylthio)-3-deazaadenosine are both effective antiviral agents. More, 3-deazaadenosine can also block chemotaxis by neutrophils and macrophages.

PHS-6040
(Rev. 10-76)

Project Description:

Adenosylhomocysteine (AdoHcy) is the product of biological methylation reactions that utilize adenosylmethionine (AdoMet) as the methyl donor. AdoHcy itself was first isolated and characterized by Cantoni and Scarano. As far as is known, the main pathway for the metabolism of AdoHcy in eukaryotes is its hydrolysis to adenosine and L-homocysteine by an enzyme, AdoHcy hydrolase, first described in rat liver by de la Haba and Cantoni. The reaction is reversible, with equilibrium far in the direction of synthesis:

$$\text{AdoHcy} \underset{H_2O}{\overset{\text{hydrolase}}{\rightleftharpoons}} \text{Adenosine + L-Homocysteine}$$

enzymatically

Many biological substances are methylated by AdoMet, such as biological amines, proteins, amino acids, lipids, carbohydrates, histones, myosine, DNA, and RNA. Interesting as a rule, almost all of the enzymes methylases involved are sensitive to product inhibition by AdoHcy.

Recently, much attention has been focused on AdoHcy per se as a biological regulator of methylation reactions, and many analogs of AdoHcy have been synthesized and their efficacies as inhibitors of biological methylations studied. We are combining a study of the molecular properties of AdoHcy hydrolase and its regulation by effectors with a detail pharmacological exploration of its perturbation by *in vivo* and *in vitro* potent inhibitors e.g. 3-deazaadenosine and 5'-deoxy-5'-(isobutylthio)-3-deazaadenosine the biological consequences of such a perturbation may be of paramount medical importance as well as yielding knowledge on the biochemical intricacies of AdoHcy and AdoMet.

AdoHcy hydrolase from calf liver was purified to homogeneity by crystallization. The purified enzyme exhibited one single component in polyacrylamide gel electrophoresis. But by ampholine gel electrophoresis, two isoelectric focusing variants were observed, with pI values at 5.8 and 6.0. When subjected to polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate, one major subunit with a molecular weight of 60,000 was found; five other minor subunit variants were also observed, with molecular weights ranging between 50,000 and 57,000. These minor subunit variants comprised approximately 15% of the total protein applied. The molecular weight of the native enzyme was estimated to be 237,500 by gradient gel electrophoresis and the native enzyme is probably composed of four subunits, each with a molecular weight of not more than 60,000. Amino acid analyses of the purified enzyme revealed the presence of 1.2 residues of glucosamine/mol of enzyme, in addition to all of the common amino acids. The presence of enzyme-bound NAD was confirmed, probably 1 NAD molecule bound/enzyme subunit. In addition to adenosine, 3-deazaadenosine was found to be an effective substrate as well in the direction of synthesis.

The effects of several analogs of AdoHcy on biological systems have been studied. It was found that 3-deazaadenosine exerts a powerful antiviral

effect on the onocogenic transformation by Rous sarcoma virus and its infectivity in chick embryo cells. Moreover, 3-deazaadenosine is able to reverse the malignant transformation induced by Rous sarcoma virus, and to restore normal growth inhibition patterns. A variety of other viruses can also be controlled by 3-deazaadenosine, and chief among them is the influenza virus. A new analog of AdoHcy, observed by us, 5'-deoxy-5'-(isobutylthio)-3-deazaadenosine, has been found to have a selective antiviral activity against Rous sarcoma virus and Gross murine leukemia virus.

In addition to its antiviral effects, 3-deazaadenosine is also a potent inhibitor of chemotaxis exhibited by neutrophils and macrophages, and phagocytosis by macrophages is blocked as well.

The biological effects of 3-deazaadenosine and 5'-deoxy-5'-(isobutylthio)-3-deazaadenosine can be attributed as a working hypothesis two mechanisms: 1) Its ability to inhibit AdoHcy hydrolase, inhibition of which would result in a change in a ratio of AdoMet/AdoHcy, which could affect methylation reactions required for biochemical processes. A perturbation in the ratio of AdoMet/AdoHcy has indeed been observed in chick embryo cells, hepatocytes, and organs of rats treated with 3-deazaadenosine. 2) A direct inhibition of methylases which utilize AdoMet, by intrinsic similarity to AdoHcy as in the case of 5'-deoxy-5'-(isobutylthio)-3-deazaadenosine, or by forming 3-deaza-AdoHcy as in the case of 3-deazaadenosine.

The possible use of analogs and/or inhibition of AdoHcy as chemotherapeutic agents will continue to be explored and the potential exploitation of AdoHcy hydrolase as a biological target in chemotherapeutic schemes will be explored. However, the implication of biological methylation in chemotaxis and phagocytaxis is evidenced by the inhibition inhibited by 3-deazaadenosine is going to elucidate immunological responses by cells; and will be quite exciting in time to come.

Publications:

Chiang, P. K., Richards, H. H. and Cantoni, G. L.: S-Adenosyl-L-homocysteine hydrolase. Analogs of S-adenosyl-L-homocysteine as potential inhibitors. Molec. Pharmacol. 13: 939-947, 1977.

Chiang, P. K., Cantoni, G. L., Ray, D. A. and Bader, J. P.: Reduced levels of adenosine deaminase in chick embryo fibroblasts by Rous sarcoma virus. Biochem. Biophys. Res. Commun. 78: 336-342, 1977.

Richards, H. H., Chiang, P. K. and Cantoni, G. L.: Adenosylhomocysteine hydrolase: crystallization of the purified enzyme and its properties. J. Biol. Chem., in press.

Bader, J. P., Brown, N. R., Chiang, P. K. and Cantoni, G. L.: 3-Deazaadenosine an inhibitor of adenosylhomocysteine hydrolase inhibits reproduction of Rous sarcoma virus and transformation of chick embryo cells. Virology, in press.

Chiang, P. K., Cantoni, G. L., Bader, J. P., Shannon, W. M., Thomas, J. and Montgomery, J. A.: Adenosylhomocysteine hydrolase inhibitors: synthesis of 5'-deoxy-5'-(isobutylthio)-3-deazaadenosine and its effect of Rous sarcoma virus and Gross murine leukemia virus. Biochem. Biophys. Res. Commun. 82: 417-442, 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00932-06 LGCB

PERIOD COVERED

October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Muscle Differentiation in Tissue Culture

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	G. L. Cantoni	Chief, Lab. Gen. Comp. Biochem.	LGCB NIMH
OTHER:	B. W. Uhlendorf	Res. Microbiologist	LGCB NIMH
	J. K. Chen	Sr. Staff Fellow	LGCB NIMH

COOPERATING UNITS (if any)

R. S. Adelstein, Chief, Sect. Molecular Cardiology, NHLI

LAB/BRANCH

Laboratory of General and Comparative Biochemistry

SECTION

Section on Proteins

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4-1/3	2-1/3	2

CHECK APPROPRIATE BOX(ES)

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Myogenesis *in vitro* has been much studied as a model of cellular differentiation. The formation of multinucleated non-proliferating electrically active and contractile myotubes by fusion of undifferentiated, proliferating myoblasts is accompanied by the appearance of muscle specific proteins, such as creatine kinase and myosin, and by the arrest of DNA synthesis. By manipulating the environmental conditions of a cloned line of myoblasts we are able to study these morphological and biochemical events. In addition, we hope to ellucidate the nature of the unknown cue(s) that signal the cell to proceed along the path of differentiation rather than the path of continued proliferation.

PHS-6040
(Rev. 10-76)

Projection Description:

Cellular differentiation is being studied. As experimental object, we are using L5/A10, a cloned, permanent cell line derived from rat skeletal muscle. By appropriate manipulation of the cultural conditions, these myoblasts can be programmed to differentiate into myotubes in a completely reproducible manner over a period of 7 days, and then, into spontaneously contracting fibers exhibiting cross-striations in 3 more days.

On the basis of observable changes in this system, we have divided the differentiation process into 3 sequential stages: the proliferative, prefusion stage (stage I), the fusion stage (stage II), and the maturation stage, during which the myotubes develop sarcoplasmic reticulum, (stage III). We have identified or characterized several events which occur during each of these stages.

During stage II we have found, as have others, that the degree of fusion, expressed as a percentage of the nuclei that are in myotubes, gradually, decreases on serial passaging of the cells. Analysis of our data shows that a major component of this decrease is due to a change in the population of nonfusing cells, namely, that under fusion-permissive conditions (but not under growth conditions) the myoblasts are able to proliferate to increasingly larger numbers with increasing passage.

In our system, the plant lectin, Con A, at 3-5 γ/ml, is capable of inhibiting the differentiation process without having an appreciable mitogenic effect, as it has on other cell types. Moreover, we have found that Con A when present during stage I, only (days 1-4) is fully active in preventing events such as, fusion and CPK production, both of which normally occur during stage II (days 4-7). Con A is not inhibitory when it is applied from the beginning of stage II. Thus, Con A is inhibiting a commitment process which occurs during stage I. The lectin does not, however, inhibit the termination of DNA synthesis which normally marks the end of stage I.

Drs. Adelstein and Scordilis have characterized the myosin light chains and the light chain kinases in extracts of cells grown under growth-permissive, fusion-nonpermissive conditions, and of cells in stages II and III. A total of about 90 grams of cells were produced for this study. The results show a developmental sequence in regard to the myosin light chain molecular weights and the light chain kinase molecular weights and Ca^{++} dependence. Under growth conditions the light chain kinase does not require Ca^{++}; at the end of stage II no light chain kinase is detected and at the end of stage III the kinase is Ca^{++} dependent.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00933-04 LGCB

PERIOD COVERED

October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Study of Adenosylmethionine Synthetase

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	G. L. Cantoni	Chief, Lab. Gen. Comp. Biochem.	LGCB NIMH
OTHER:	P. K. Chiang	Sr. Staff Fellow	LGCB NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of General and Comparative Biochemistry

SECTION

Section on Proteins

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
5/6	5/6	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purification and characterization of the properties of adenosylmethionine (AdoMet) synthetase are being pursued further. AdoMet synthase from yeast are inhibited by cycloleucine, L-methionine methyl ester, L-methionine ethyl ester, and L-methionine t-butyl ester. 3-Deazaadenosylmethionine stimulates the tripolyphosphatase (PPPase) activity of AdoMet synthetase while it also enhances the steady state rate of AdoMet synthetase. Although guanosylmethionine and cytidylmethionine can also stimulate the PPPase to a lesser extent, are ineffective in enhancing the steady state rate of AdoMet synthetase.

HS-6040
(Rev. 10-76)

Projection Description:

Ever since its discovery by Giulio L. Cantoni, adenosylmethionine (AdoMet) is receiving increasing attention with time. The physiological role played by AdoMet is undisputedly important and diversified. Three substitutents are attached to the sulfonium atom by equivalent bonds, imparting the AdoMet molecule, its unique versatility. Apparently, no other biological compound functions in as many types of biochemical reactions: 1) as a donor of its methyl group in many and the majority of transmethylation reactions; 2) as donor of aminobutyric side chain; 3) as donor of a propylamine moiety after decarboxylation; 4) as an allosteric effector in enzymatic reactions.

In the field of mental health, chief among the many methylated products are methylated catecholamines, methylated catechols and other methylated biogenic amines. A biochemical basis for affective disorders and schizophrenia has been suggested, especially regarding methylated biogenic amines and this hypothesis will undoubtedly receive closer scrutiny by scientists and clinicians.

AdoMet itself is synthesized by an enzyme, AdoMet synthetase (ATP: L-methionine adenosyltransferase) (E.C. 2.5.1.6). This enzyme has been purified from yeast to homogeneity for the first time by us. We reported that there are two forms (isozymes) of AdoMet synthetase. Since then, two forms of AdoMet synthetase from mammalian tissues have also been reported by others, thus confirming our initial report. However, successful attempts to purify AdoMet synthetase from mammalian sources have not been reported. In view of physiological implications, it may be desirable to explore ways to purify AdoMet synthetase from mammalian sources, especially regarding the biochemical roles played by these two isozymes. Pathologically, variations in the level of AdoMet synthetase have been reported in children with hypermethionemia, in patients with leukemia and acute schizophrenia.

Meanwhile, the kinetic properties of AdoMet synthetase II are still being characterized by us. We found that cycloleucine is not the only good inhibitor for AdoMet synthetase. L-Methionine methyl ester, L-methionine ethyl ester and L-methionine t-butyl ester are good inhibitors also. Other methionine analogs will be explored to see if more potent inhibitors can be found.

Interestingly, 3-deazaadenosylmethionine (3-cAdoMet), an analog of AdoMet, is a stable and good activator of the tripolyphosphatase (PPPase) activity of the AdoMet synthetase. In contrast to AdoMet itself, 3-cAdoMet can also stimulate the steady state rate of AdoMet synthetase by about 10% without altering the kinetic profile of the Michaelis curve. Other AdoMet analogs, guanosylmethionine and cytidylmethionine can also stimulate to a lesser extent the PPPase activity of AdoMet synthetase, while no such effect can be observed with inosylmethionine. No stimulatory effect on the steady state rate of AdoMet synthetase is observed with guanosylmethionine, cytidylmethionine and inosylmethionine. These three analogs are also found to be very unstable in solution.

Publication:

Chiang, P. K. and Cantoni, G. L.: Activation of methionine for transmethylation. Purification of the S-adenosylmethionine synthetase of Bakers' yeast and its separation into two forms. J. Biol. Chem. 252: 4506-4513, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00934-06 LGCB

PERIOD COVERED

October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Studies on the Biochemical Basis of Narcotic Drug Action

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	W. A. Klee	Research Chemist	LGCB NIMH
OTHER:	C. Zioudrou	Visiting Scientist	LGCB NIMH
	M. Knight	Chemist	LGCB NIMH
	M. Nirenberg	Chief, Lab. Biochem. Genetics	LBG NHLI
	A. E. Jacobson	Research Chemist	LC NIAMDD
	K. C. Rice	Research Chemist	LC NIAMDD
	M. Mata	Visiting Fellow	LDN NICHHD
	H. Gainer	Head, Section on Functional Neurochem.	LDN NICHHD

COOPERATING UNITS (if any)

Laboratory of Biochemical Genetics, NHLI
Laboratory of Chemistry, NIAMDD
Laboratory of Developmental Neurobiology, NICHHD

LAB/BRANCH

Laboratory of General and Comparative Biochemistry

SECTION

Section on Proteins

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3	2	1

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A cell line of neural origin (NG108-15) which carries many opiate receptors is used to study the mechanism of action of morphine and morphine-like peptides (endorphin). These materials exert a receptor mediated dual regulation of adenylate cyclase; a rapid inhibition of activity upon which is superimposed a subsequent increase in the amount of total expressible enzyme activity. This, homeostatic, mechanism may account for tolerance to and dependence upon the opiates. The enkephalins were found to be much more potent than their presumed precursors, the endorphins as mediators of dual regulation. A new class of opioid peptide, the exorphins has been discovered which is derived from food proteins *via* the normal digestive process. The exorphins may provide a new biochemical link between eating, emotional state and psychiatric illness.

PH-6040
(Rev. 10-76)

Projection Description:

In our studies of the mechanism of morphine action we have continued to exploit properties of the neuroblastoma x glioma cell line NG108-15. This hybrid cell has a large number of opiate receptors which are functionally coupled to the enzyme, adenylate cyclase, as inhibitors, as we had demonstrated earlier. These cells become addicted to morphine and other opiates as a result of being cultured in their presence for 12 hours or longer. The addictive process is a homeostatic mechanism which serves to maintain cellular cyclic AMP levels near their normal values. The acute effects of opiates are to inhibit adenylate cyclase and thereby rapidly lower cAMP levels; the chronic adaptation is due to a gradual expansion in the amount of enzyme activity which serves to maintain normal cAMP levels even in the continued presence of morphine. The increased enzyme activity is due, at least in part, to changes in the regulatory properties of the pre-existing enzyme rather than to an increase in the number of enzyme molecules in the cell.

Peptides with morphine-like activity have recently been characterized by Hughes and others after purification from brain extracts. These peptides, the enkephalins and endorphins, all seem to be fragments of the pro-hormone β-lipotropin and can thus be considered neurohormones. We have tested many peptides of this class with adenylate cyclase prepared from NG108-15 cells and find them to be among the most potent, receptor mediated, inhibitors known for this enzyme. Furthermore, when cultures of NG108-15 cells are exposed to large amounts of these peptides for a number of days, they become addicted in exactly the manner found for morphine and the other opiates. Our data, to date, show that the only difference in action between the enkephalins and the classical opiates is a much shorter duration of action of the peptides due, perhaps, to proteolytic degradation. We have initiated a study of the peptidases, present in brain, which are concerned with the synthesis, from larger precursors, and the degradation of enkephalin. The membrane bound aminopeptidase which is apparently responsible for the inactivation of enkephalin has been found to be functionally coupled to opiate receptors since receptor bound peptide appears to be rapidly degraded. This study has also led to the finding that the antibiotic, puromycin, is a potent inhibitor of the aminopeptidase (much more so than is bactracin or other peptides) and raises the possibility that some of the behavioral effects of puromycin after intracerebroventricular administration may be due to a potentiation of neuropeptide action rather than to inhibition of protein synthesis. Efforts are currently underway to purify the aminopeptidase as well as those adenopeptidases involved in enkephalin biosynthesis.

Our studies of the specificity of the opiate receptor for enkephalin analogues showed that, although the structural requirements for optimal interaction are fairly strict, active peptides can be found which differ appreciably in structure from enkephalin. In view of the extraordinary potency of the natural peptide, analogues which are one or two orders of magnitude weaker are still very potent legands for the receptor and inhibitors of adenylate cyclase. We were thus led to consider the possibility that peptides with enkephalin-like activity might be derived from food proteins *via* the normal digestive process. Our attention was initially focused upon wheat gluten since Dohan has suggested

a causal relationship between gluten intake and schizophrenia. We reasoned that, if gluten were converted to behaviorally active peptides in the body, a plausible biochemical mechanism for Dohan's observations would be established. Our experiments have shown that the stomach proteinase, pepsin, effectively converts wheat gluten into a mixture of peptides some of which have naloxone reversible, opiate activity in the adenylate cyclase assay and in the mouse vas deferens. We have purified some of these peptides to a state approaching homogeneity by a succession of chromatographic procedures. We choose to call these materials exorphins, since they are opioid peptides derived from exogenous materials. Some other proteins, such as α-casein for example, also serve as sources of exorphins and we are purifying some of these substances as well. The exorphins are relatively resistant to trypsin and chymotrypsin but are inactivated by less fastidious proteinases such as pronase. They are hydrophobic in nature and may therefore be absorbed from the gastrointestinal tract as such. Some of the exorphins appear to be more potent than morphine and to approach the extraordinary potency of the endogenous opioid peptides. Only very small amounts would therefore need to reach the brain for these substances to have significant effects. The possible role of exorphins in the regulation of feeding behavior, the relationship of appetite and emotional state and in psychiatric illness remains to be clarified.

The receptor coupled adenylate cyclase of NG108-15 membranes provides a rapid, convenient and sensitive assay for both the agonist and antagonist properties of opiates. Agonists such as morphine inhibit the enzyme activity whereas antagonists such as naloxone block the action of agonists. Mixed agonists, such as nalorphine, inhibit adenylate cyclase partially, and reverse the inhibitory action of agonists, partially. The method is being used by us to study the mechanisms of opiate antagonist action and to follow the purification of exorphins. In addition we have used this method to measure pituitary endorphin levels of rats as a function of physiological state. We found that there is an interaction between vasopressin and pituitary endorphin in that the two are apparently released concomitantly.

Work is in progress on the development of new affinity ligands for the endorphin receptor, based both on peptides and alkaloid type ligands. These materials should be important aids in our efforts to isolate the receptors in purified form and serve as useful pharmacological probes as well.

Publications:

Sharma, S. K., Klee, W. A. and Nirenberg, M.: Opiate dependent modulation of adenylate cyclase. Proc. Natl. Acad. Sci. U. S. A. 74: 3365-3369, 1977.

Jacquet, Y. F., Klee, W. A., Rice, K. C., Iijima, I. and Minamikawa, J.: Stereospecific and non-stereospecific effects of (+) and (-) morphine: evidence for a new class of receptors? Science 198: 842-845, 1977.

Mata, M. M., Gainer, H. and Klee, W. A.: Effect of dehydration on the endogenous opiate content of the rat neuro-intermediate lobe. Life Sci. 21: 1159-1162, 1977.

Rice, K. C., Klee, W. A., Aceto, M. D., Swain, H. H. and Jacobson, A. E.: Potential long acting opiate antagonists: the preparation, pharmacological activity and opiate-receptor binding of N-substituted 2'-Hydroxy-5-Methyl-9α-propyl-6,7-benzomorphons. J. Pharm. Sci. 66: 193-197, 1977.

Klee, W. A., Zioudrou, C. and Streaty, R. A.: Exorphins - Peptides with Opioid Activity Isolated from Wheat Gluten, and Their Possible Role in the Etiology of Schizophrenia. In Usdin, E., Bunney, W. E. and Kline, N. S. (Eds.): Endorphins in Mental Health Research. New York, McMillan, 1978, pp. 209-218.

Knight, M. and Klee, W. A.: The relationship between enkephalin degradation and opiate receptor occupancy. J. Biol. Chem. 253: 3843-3847, 1978.

Iijima, I., Minamikawa, J., Jacobson, A. E., Brossi, A., Rice, K. C. and Klee, W. A.: Synthesis and biological activity of (+)-naloxone. J. Med. Chem. 21: 398-400, 1978.

Klee, W. A.: Dual Regulation of Adenylate Cyclase: A Biochemical Model for Opiate Tolerance and Dependence. In Fishman, J. (Ed.): The Bases of Addiction. Dahlem Workshops, Berlin, 1978, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00935-11 LGCB

PERIOD COVERED

October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Effect of Small Viruses and Their Nucleic Acids on the Biochemistry of Living Organisms

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	C. R. Merril	Sr. Res. Scientist	LGCB NIMH
OTHER:	A. S. Adhya	Geneticist	LMB NCI
	A. K. Das	Visiting Fellow	LMB NCI
	M. Gottesman	Head, Biochemical Genetics Section	LMB NCI
	J. Leavitt	Sr. Fellow, Bureau of Biologics	FDA
	M. H. Ebert	Chief, Section on Experimental Therapeutics	LCS NIMH
	E. D. Caine	Med. Officer, Section on Experimental Therapeutics	LCS NIMH

COOPERATING UNITS (if any)

Laboratory of Molecular Biology, NCI
Bureau of Biologics, FDA
Laboratory of Clinical Science, NIMH

LAB/BRANCH

Laboratory of General and Comparative Biochemistry

SECTION

Section on Proteins

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3	1	2

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Assays for the purine phosphoribosyltransferase enzymes were developed to test for prokaryotic gene expression in human cells. These assays uncovered a greater complexity in the bacterial purine pathways than had been expected. This complexity may explain the results obtained when infecting mammalian cells with the bacterial virus, lambda gpt, carrying prokaryotic genes concerned with purine metabolism. Evidence has been obtained which indicates that this virus may not contain a structural gene but instead may contain a purine control element or a posttranslational modifier. The purine assays were also used to investigate the Gilles de la Tourette syndrome. It had been suggested that self-mutilating Tourette patients have a temperature senstive purine enzyme (hypoxanthine-guanine phosphoribosyltransferase) with an unusual isoelectrofocusing pattern. We examined 11 Tourette syndrome patients, 10 relatives and 3 normal controls. Although 3 of our cases experienced self-mutilation no consistant temperature sensitivity was observed for the HGPRT enzyme. HGPRT purified by immunoprecipitation from the patients lyzed red blood cells demonstrated normal isoelectrofocusing, and SDS molecular weight patterns, using (2D) electrophoresis techniques. The use of (2D) electrophoresis and immunoprecipitation has been extended to screen for biochemical abnormalities in Huntington's chorea and other involuntary movement disorders.

Project Description:

The use of the galactose pathways to screen for bacterial virus gene expression in whole animals continues to be hampered by the death of the animals following injection with the viral preparations. The use of chloroform extractions prior to purification by binding in CsCl gradients (to reduce the level of endotoxin in the viral preparations) has had little if any effect on the death rate. The use of the galactose enzyme assays and DNA-RNA hybridization techniques has led to the discovery of discoordinate gene expression following escape synthesis of the genes of the galactose operon of *E. coli*. The epimerase enzymes was found to have little if any increase in activity while the kinase enzyme activity went up 30-fold during escape synthesis. Paradoxically the mRNA for all enzymes increased coordinately. Most recently cyclic AMP stimulation of the galactose operon has revealed a discoordinate gene expression in which the epimerase is expressed in excess when compared to kinase activity.

Publications:

Merril, C. R., Leavitt, J., Van Keuren, M. L., Ebert, M. H., Caine, E. D.: Hypoxanthine guanine phosphoribosyltransferase (HGPRT) in Gilles de la Tourette syndrome. Neurology, in press.

Merril, C. R.: Interactions of Bacterial Viruses and Bacterial Genes with Animal Systems in Molecular Genetic Modification of Eucaryotes. In Rubenstein, I., Phillips, R. L., Green, C. E. and Desnick, R. (Eds.): Molecular Genetic Modification of Eucaryotes. New York, Academic Press, 1977, pp. 83-98.

Merril, C. R., Gottesman, M., Court, D. and Adhya, S.: Discoordinate expression of the *E. coli* gal operon after prophage lambda induction. J. Mol. Biol. 118: 241-245, 1978.

Geier, M. R., Stanbro, H. and Merril, C. R.: Endotoxins in commercial vaccines. Appl. Envir. Microbiol., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00936-15 LGCB

PERIOD COVERED

October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Homocystinuria: Methionine Metabolism in Mammals

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	S. H. Mudd	Chief, Section on Alkaloid Biosynthesis	LGCB	NIMH
OTHER:	R. Havlik	Medical Officer, Epidemiology Branch	HV	NHLBI
	M. Feinleib	Chief, Epidemiology Branch	HV	NHLBI

COOPERATING UNITS (if any)

Division of Heart and Vascular Diseases, NHLBI

LAB/BRANCH

Laboratory of General and Comparative Biochemistry

SECTION

Section on Alkaloid Biosynthesis

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
5/12	2/12	3/12

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A survey of the health status of parents and grandparents of cystathionine synthase deficient patients has been initiated. The aim is to determine whether heterozygotes for this genetic defect are at increased risk for coronary artery disease. By appropriate physician contact we have, or soon will have, disbursed questionnaires to 200-250 such families. Depending upon the incidence of coronary disease in the control population, this survey should provide at least 80% chance of detecting a statistically significant ($p<.05$) three-fold increase in the incidence of coronary artery disease among parents and/or grandparents. Work is now underway to obtain comparable data for three control groups: relatives of patients with (a) cystinosis, (b) phenylketonuria, and (c) achondroplastic dwarfism. The latter is the group of choice for several reasons and the major effort initially will focus upon this group.

PHS-6040
(Rev. 10-76)

Project Description:

During the interval since the last annual report, most of our efforts on this project have been devoted to the epidemiological study which seeks to determine whether heterozygotes for cystathionine synthase deficiency are at increased risk for early coronary artery disease. The rationale for undertaking this study was set forth in detail in the previous annual report. The pilot study mentioned there has now been completed, and changes in the questionnaire suggested on the basis of these results incorporated. For some time, we have been contacting physicians who might be in touch with families with cystathionine synthase deficient patients, and soliciting the assistance of these physicians in distribution of the questionnaire to these families, and in ensuring that a response is obtained. More than 175 physicians have been contacted in North America, and as a result approximately 200 families with at least one homocystinuric patient have been ascertained and have, or will very shortly, receive questionnaires. This number compares favorably with an estimate (based on the incidence of homozygous cystathionine synthase deficiency detected in screening programs of new-born children, the population of North America, the life expectancy of homozygotes for cystathionine synthase deficiency, and the likelihood that affected patients will be clustered in sibships) that there should be 350-400 families in North America with at least one surviving homozygous cystathionine synthase deficient patient. Outside North America, arrangements have been made to obtain rather complete coverage in England, Northern Ireland, and Australia. Each of these countries is known to have significant number of homocystinuric patients. Efforts are underway to arrange similar coverage in Ireland and New Zealand, thus completing coverage of most of the English-speaking world. Together, these countries may add more than 50 families. For several reasons we are currently not planning to cover non-English speaking areas. Thus, with sufficient follow-up effort to ensure a good response rate, it may be possible to obtain information on as many as 200 sets of parents and 400 sets of grandparents. Depending upon the exact incidence of coronary artery disease in the control population, these numbers of parents and grandparents should be enough to provide at least 80% chance of detecting a statistically significant ($p<.05$) three-fold increase in the incidence of coronary artery disease among the parents and/or grandparents.

Work has commenced also, on the data collection from one control group, composed of parents and grandparents of children with cystinosis.

In the immediate future, it is planned to systematically attempt to ensure a good response rate from among the homocystinuric families, by repeated contact with the cooperating physicians. The control group will be expanded to include about 250 families in which a child has a newly arisen mutation causing achondroplastic dwarfism. It is possible to identify such families because the mutation in question is dominant. In about 80% of families with achondroplastic dwarfism, the mutation is a new one. This is the control group of choice because: (1) a register is available of about 280 such families, (2) the condition is present at birth, but is not associated with any known predisposition to coronary artery disease, and (3) the parents and

grandparents will not be preselected as heterozygotes for any one specific mutation. One drawback with this group is that the age-distribution of the fathers will be shifted upward. Hopefully, this can be overcome by appropriate analysis of the data. Finally, tentative plans have been made to survey another control group, namely families with phenylketonuric children. This will probably be delayed until a clearer assessment is possible of the age-distribution, etc., in the first study groups.

Publications:

Freeman, J. M., Finkelstein, J. D. and Mudd, S. H.: Folate responsive homocystinuria and "schizophrenia." A defect in methylation due to deficient 5,10-methylenetetrahydrofolate reductase activity. New Engl. J. Med. 292: 491-496, 1975.

Mudd, S. H.: Cobalamin-Responsive Genetic Disorders. In Shimazono, N. and Arakawa, T. (Eds.): Nutritional Deficiency Secondary to Inborn Errors of Metabolism Its Relation to Physical and Mental Development. Japanese Panel of Malnutrition U.S.-Japan Cooperative Medical Sciences Program, Sendai, Japan, 1976, pp. 251-265.

Mudd, S. H. and Levy, H. L.: Disorders of Transsulfuration. In Stanbury, J. B., Wyngaarden, J. B. and Fredrickson, D. S. (Eds.): The Metabolic Basis of Inherited Disease--ed. 4. New York, McGraw-Hill, 1978, pp. 458-503.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00937-13 LGCB

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Transsulfuration in Higher Plants

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. Giovanelli	Research Chemist	LGCB NIMH
	A. H. Datko	Botanist	LGCB NIMH
	S. H. Mudd	Chief, Section on Alkaloid Biosynthesis	LGCB NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of General and Comparative Biochemistry

SECTION
Section on Alkaloid Biosynthesis

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3-7/12	2-10/12	9/12

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Work on the mechanism of control of the biosynthesis of methionine and other members of the aspartate family of amino acids has concentrated on *in vivo* studies to outline physiologically important regulatory patterns. (1) Lysine and threonine in combination inhibited growth of *Lemna* completely at concentrations which individually had little effect on growth. The inhibition was relieved by methionine, homoserine or homocystine. These observations are consistent with the combination of lysine and threonine exerting concerted feedback control of the synthesis of the aspartate family of amino acids as an early step (e.g. that catalyzed by aspartokinase) that is required for synthesis not only of lysine and threonine, but also of methionine. (2) Growth of *Lemna* with $^{35}SO_4^{2-}$ either in the presence or absence of supplementary methionine demonstrated that *de novo* synthesis of the sulfur moiety of methionine can be reduced to less than 25% of its normal rate by feedback control by methionine, and that the conversion of cysteine to cystathionine (catalyzed by cystathionine γ-synthase) is the probable site of this control. (3) A potent inhibition of growth of *Lemna* by the aminoethoxy and methoxy derivatives of vinylglycine was demonstrated. Present data are consistent with the derivatives acting primarily by inhibition of β-cystathionase. The derivatives therefore promise to be valuable tools for specific inhibition of the transsulfuration pathway.

Project Description:

Our previous work has demonstrated that in the two plants examined (the lower plant *Chlorella* and the higher plant *Lemna*), the transsulfuration pathway is the dominant route for biosynthesis of methionine. In *Lemna*, direct sulfhydration also contributes a small proportion (5-10%) of the total methionine synthesized. Our major objective is to determine the mechanism of regulation of these two pathways, as well as that of the other aspartate family amino acids (lysine, threonine and isoleucine).

In our research this year, we have extended our knowledge of certain aspects of the physiology of growth of *Lemna perpusilla*, and have continued *in vivo* experiments with this plant to study the physiologically important patterns of regulation of the synthesis of methionine and other amino acids of the aspartate family. Details of our major findings are:

(1) We have completed studies to define conditions for optimal growth of *Lemna*, and to determine the limits within which temperature, pH, CO_2 or sucrose concentration, light, and inorganic nutrient composition must be controlled in order to avoid inadvertent effects on growth. This work has provided a standard set of conditions for the rapid and reproducible growth of *Lemna* both photoautotrophically and heterotrophically.

(2) *Lemna* reproduces vegetatively by producing daughter fronds alternatively from two pockets on either side of the (mother) frond. This process is repeated by the daughter fronds, usually while still attached to the mother frond. Ultimately the group of fronds (i.e. colony) separates into a daughter colony and a mother colony containing the original mother frond. A better understanding of certain aspects of this growth process would be of value in the design and interpretation of our experiments on the regulatory patterns of amino acid biosynthesis. The following properties of the growth process in *Lemna perpusilla* were determined:

(a) Mother colonies that are selected at the same stage in the division cycle produced up to 7 daughter colonies in a highly synchronous fashion.

(b) Each mother colony was shown to have a limited life span, producing on the average 18 daughter colonies.

(c) Colonies were labeled to isotopic equilibrium with $^{35}SO_4^{2-}$, transferred to nonradioactive medium, and the distribution of ^{35}S between mother and subsequent daughter colonies determined. Mother colonies were found to donate the following percentages of their radioactivity to succeeding daughter colonies: first (24%), second (20%), third (19%) and fourth (16%). Radioactivity was predominantly insoluble in cold TCA, and is presumed to be mainly protein methionine plus protein cyst(e)ine. The results suggest that production of daughter colonies is associated with a significant turnover of protein methionine and protein cyst(e)ine in mother colonies. Furthermore, a significant proportion of the cellular organic sulfur of daughter colonies is provided by the mother colony, so that daughter colonies may not synthesize all of their

organic sulfur *de novo* from inorganic sulfate.

(3) We have previously shown with spinach and corn seedlings that the bacterial toxin rhizobitoxine (a derivative of vinylglycine) inhibits the transsulfuration pathway by an active-site-directed irreversible inhibition of β-cystathionase. Sufficient amounts of the aminoethoxy and methoxy derivatives of vinylglycine (but not of rhizobitoxine) became available to make it practical to extend these studies to *Lemna*. Growth of *Lemna* was found to be extremely sensitive to both the methoxy derivative (50% inhibition at approxamately 8 nM), and the aminoethoxy derivative (50% inhibition at approximately 40 nM). These concentrations are at least two orders of magnitude less than those reported by other workers to be required for comparable inhibition by these derivatives of other physiological processes (e.g. synthesis of protein and ethylene) in plants. Neither the corresponding saturated derivatives nor vinylglycine at concentrations up to 3000 nM were inhibitory. Inhibition by the aminoethoxy derivative was relieved by methionine or homocystine but not by homoserine, and inhibition by the methoxy derivative was relieved by methionine (other compounds not investigated). These preliminary data are consistent with the derivatives inhibiting growth chiefly by inhibition of β-cystathionase. These derivatives of vinylglycine therefore show promise as potent and specific inhibitors of the transsulfuration pathway.

(4) Studies on the growth effects of amino acids that are suspected of being feedback regulators can freqently provide valuable clues on overall regulatory patterns. The following example illustrates the kind of information that we have obtained in an experiment with *Lemna*. Lysine and threonine in combination were found to inhibit growth completely at concentrations which individually had little effect on growth. The inhibition was relieved by methionine or homocysteine. These observations are consistent with the conclusion drawn from results obtained with other organisms that these amino acids together exert concerted feedback control at an early step in the synthesis of the asparate family of amino acids. Concerted inhibition at this step inhibits not only the synthesis of lysine and threonine but also that of methionine. In the presence of lysine and threonine in combination, growth therefore becomes dependent upon methionine or its immediate precursor, homocysteine.

(5) Experiments have been completed to determine whether *de novo* synthesis of the sulfur moiety of methionine is subject to feedback control by methionine. *Lemna* was grown to isotopic equilibrium either with $^{35}SO_4^{2-}$, or with $^{35}SO_4^{2-}$ supplemented with methionine. If methionine exerts feedback control, then supplementary methionine should be used in preference to that synthesized *de novo* from $^{35}SO_4^{2-}$, and the presence of supplementary methionine should therefore inhibit the incorporation of $^{35}SO_4^{2-}$ into methionine and its products. It was found that supplementary methionine reduced the incorporation of $^{35}SO_4^{2-}$ into methionine and its products to less than 25% of that observed with $^{35}SO_4^{2-}$ alone, without affecting the incorporation of $^{35}SO_4^{2-}$ into cysteine and its products. The relatively small amounts of ^{35}S accumulating in cystathionine and homocysteine were also not significantly affected by methionine supplementation. These combined observations demonstrate that *de novo* synthesis of the sulfur moiety of methionine can be reduced to less than 25% of its normal rate

by feedback control by methionine and suggest that the conversion of cysteine to cystathionine (catalyzed by cystathionine γ-synthase) is the major site of this control.

Future work will be continued along the following lines:

(a) Experiments analogous to those described in (5), but with ^{14}C-sucrose replacing $^{35}SO_4^{2-}$, are in progress to determine whether the carbon moieties of methionine are also subject to feedback control by methionine, and if so, at what site(s) control occurs.

(b) The mechanism whereby supplementary methionine controls *de novo* incorporation of sulfur (and possibly also that of C4 moiety) into cystathionine will be examined. Synthesis of cystathionine may be controlled either by the activity of cystathionine γ-synthase, or by the concentration of *O*-phosphohomoserine, the α-aminobutyryl donor for cystathionine γ-synthase in plants. Since the first mechanism requires that the concentration of *O*-phosphohomoserine increase during feedback inhibition by methionine, while the second requires that the concentration of *O*-phosphohomoserine decrease, the physiological significance of these two mechanisms can be distinguished by determining the effects of feedback inhibition by methionine on the concentration of *O*-phosphohomoserine.

(c) Compounds other than methionine itself (e.g., S-adenosylmethionine, methionyl-tRNA and S-methylmethionine sulfonium) may be the physiological effector for the feedback control observed with supplementary methionine. As a first step in identifying the effector compound(s) we plan to correlate the tissue concentration of potential effectors with the extent of feedback control.

(d) The overall rate of synthesis of methionine and other amino acids of the aspartate family will be determined in *Lemna* growing in the presence of selected amino acid intermediates. These experiments can indicate the extent to which key reactions in the synthesis of methionine and other amino acids of the aspartate family are saturated with substrate. Such information is valuable for two main reasons. First, it helps to distinguish between the two major mechanisms of regulation - i.e., by substrate concentration and by enzyme activity. Thus, in general, enzyme reactions that operate well below substrate saturation are more efficiently regulated by changes in substrate concentration, whereas those that operate near substrate saturation are more efficiently regulated by changes in enzyme activity. Second, in terms of our long-range goal of increasing the content of methionine and other essential amino acids in plants, such information will allow our efforts to be focused on increasing the concentration of those intermediates that are limiting the overall rate of synthesis of these amino acids.

(e) Our studies over the last few years have concentrated on outlining the major physiologically important pathways and control mechanisms for methionine biosynthesis in intact tissues. These *in vivo* studies continue to raise many interesting questions that we hope to pursue at the molecular level.

Publications:

Giovanelli, J., Mudd, S. H. and Datko, A. H.: Homocysteine biosynthesis in green plants. Physiological importance of the transsulfuration pathway in *Chlorella sorkiniana* growing under steady-state conditions with limiting sulfate. J. Biol. Chem., in press.

Datko, A. H., Mudd, S. H., Giovanelli, J. and Macnicol, P. K.: Sulfur-containing compounds in *Lemna perpusilla* 6746 grown at a range of sulfate concentrations. Plant Physiol., in press.

Datko, A. H., Mudd, S. H., Macnicol, P. K. and Giovanelli, J.: A phytostat for the growth of *Lemna* in semicontinuous culture with low sulfate. Plant Physiol., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH00981-13-LNB

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Analysis of the Macromolecular Structure of Nerve Membrane During Excitation

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
6.5	4.0	2.5

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Studies of the macromolecular structure of the nerve membrane involved in excitation were carried out by using the following four different methods. (1) A new electrophysiological method which combines the use of a real-time spectrum-analyzer with chemical nerve stimulants. (2) The method of radioactively labeling the proteins released from the interior of the squid giant axon under intracellular perfusion. (3) The method of radioactively labeling intact, excitable axons followed by SDS-acrylamide gel electrophoresis of axonal proteins. (4) The standard biochemical method of subcellular fractionation of nerve membrane homogenate. All four methods of investigation were found to be extremely useful in elucidation of the properties of proteins at excitable membrane sites.

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(Rev. 10-76)

1. The real-time spectrum analyzer used was purchased from Nicolet Scientific Corp., Northvale, N.J. With this machine, the existence of a new type of electric response in the nerve membrane was revealed. The amplitude of these responses are extremely small (1-30 μV in amplitude). Since these responses are fired repetitively under the influence of various chemical stimulants, it was found possible to prove their existence by determining the signal-spectra through signal averaging. The chemical stimulants used include (i) scorpion venoms, (ii) veratridine, (iii) glutaraldehyde, (iv) osmium tetroxide, (v) 4-dimethylaminopyridine, (vi) aminopyridine, (vii) rose bengal combined with exposure to strong light (for photo-oxidizing histidine groups in proteins), and (viii) salt solutions with a reduced Ca-ion concentration. These newly discovered electrical responses are named "periodic miniature responses."

The general physiological properties of these responses are as follows: (i) At 20° C, the frequency of these responses is in the range between 100 and 200 Hz; lowering of the temperature enhances the amplitude and decreases the frequency. (ii) Tetrodotoxin (TTX) suppresses these responses at concentrations of about 10^{-8} M. (iii) Intracellular injection of tetraethylammonium (TEA) salt lowers the frequency. (iv) The low-frequency responses produced in TEA-treated axons can be readily suppressed by TTX. (v) With scorpion venoms, 4-dimethylaminopyridine and low Ca, these responses are produced without being accompanied (or preceded) by a fall in the membrane potential.

The physiological significance of these findings are as follows. In the membrane of peripheral nerve fibers, there are receptor sites consisting of protein molecules. These sites can be electrophysiologically activated by various chemical stimulants including reagents which alter the primary structure of proteins. When the density of the sites activated is very low, there is no electric interaction between the activities at different sites; the periodic miniature responses are the signs of the activities taking place in non-interacting membrane receptor sites. We regard full sized action potentials (about 110 mV in amplitude) as representing activation of all the receptor sites in the membrane simultaneously.

We believe that the experimental findings described above have opened up a new field of investigation in membrane physiology.

2. Isolation of protein from nerve membrane. In our continuing studies of the role of axonal proteins in maintaining the excitability of nerve, we have, in the past year, obtained the following findings. The proteins in the perfusate collected from intracellularly perfused squid giant axons were analyzed after being labeled with radioactive ^{125}I-Bolton-Hunter reagent. The rate of protein release into the perfusate was found to be increased by the following electrophysiological manipulations of the axons: (i) repetitive electrical stimulation at 60 Hz in axons perfused with standard KF containing solution or at 1/8 Hz in axons perfused with tetraethylammonium (TEA) containing solution, (ii) perfusion with 4-aminopyridine solution which induces spontaneous electrical activity in

the axon, and (iii) depolarization of the axon induced by raising the external potassium concentration. SDS-polyacrylamide gel electrophoresis of the proteins released under these conditions yielded molecular weight profiles different from those of the extruded axoplasmic proteins. These observations indicate that there exists, in close association with the axonal membrane, a particular group of proteins of which the solubility is readily affected by changes in the state of the membrane.

In addition, intracellular perfusion with a high pH solution increased the rate of protein release markedly, while a low pH solution did not. Replacement of K ion in the intracellular perfusion solution with Na, Cs, or tetraethylammonium (TEA) ions enhanced the protein release. The molecular weight distribution of proteins released under these conditions showed patterns similar to that of the protein released during nerve excitation. The effect of high pH and of monovalent cation replacement was interpreted in terms of the protein solubilizing power of cations and the hydration state of the protein attached to the internal surface of the axonal membrane. Based on this interpretation, we have explained the prolongation of action potentials from a macromolecular point of view.

Finally, in addition to the protein analysis on SDS polyacrylamide gels, we have analyzed the axonal proteins on isoelectric focusing gels. Currently, efforts are under way to analyze these proteins by the method of two-dimensional electrophoresis, in which the proteins are separated by isolelectric focusing in the first dimension and by SDS polyacrylamide electrophoresis in the second dimension.

3. Protein phosphorylation in the squid axon. Proteins in the squid giant axon were labeled with ^{32}P by in vitro incubation of isolated axoplasm with radioactive [γ-^{32}P] adenosine triphosphate (ATP) and separated by polyacrylamide sodium dodecyl sulfate (SDS) gel electrophoresis. The two major phosphorylated regions on the gel had molecular weights of 400,000 and 200,000 daltons. The phosphorylation of these proteins is stimulated by Mg^{2+}, inhibited by Ca^{2+}, and unaffected by 10^{-7} -10^{-5} M cyclic nucleotides. These two peaks appear to be neurofilament proteins of squid axoplasm. The same set of proteins was phosphorylated in the axoplasm regardless of whether the [γ-^{32}P]-ATP was applied in situ intracellularly or extracellularly. These results suggest that ATP in the extracellular space is, by some ATP-translocation mechanism, utilized in the process of intracellular phosphorylation. Measurements of the apparent influx of ATP across the squid axon membrane yielded results consistent with the view that ATP in the extracellular fluid could be transported into the axoplasm.

4. Subcellular approach. The techniques of subcellular fractionation of crab nerve have been developed and have been shown to be capable of yielding a fairly pure fraction of axonal membrane fragments (vesicles). The binding of calcium to these membranes has yielded some preliminary evidence indicating that sodium competes for the bound calcium better than does potassium. This has been tentatively interpreted to mean that the

calcium is binding to a variety of sites on the membrane, most of which bear little relation to excitability. In order to reduce these difficulties with non-specific binding, radioactive tetrodotoxin binding studies are under way. It is hoped that these studies will yield a molecular map of the patch on the membrane which undergoes conformational changes eliciting the action potential.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00982-11 LNB

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Sensory-motor Integration in the Primate Visual System

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	B.J. Richmond	Staff Associate	LNB NIMH
	S.J. Judge	Visiting Fellow	LNB NIMH
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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
6.0	4.5	1.5

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

We investigated how cells in the visual system of the monkey are influenced by rapid eye movements. We found that in the visual cortical pathway of the monkey, stimuli before and after the rapid movement act to reduce the sensitivity of the system to that movement, but that the physiological mechanisms for the stimuli preceding and following the eye movement are quite different. Parallel experiments on perception of the same stimuli by human observers produced time courses of perceptual masking comparable to the effects seen at the single cell level in the monkey. In the other visual pathway, that to the superior colliculus, a visual masking effect has also been identified so that the masking effect is a general one in both visual pathways.

The role of the superior colliculus in the initiation of eye movement was also investigated. The normal monkey makes many eye movements and the smaller ones of less than 15° predominate. Following ablation of the colliculus, while monkeys were still able to detect visual stimuli in the central visual field and make eye movements to them, the monkeys made fewer spontaneous small saccades. In addition, they were no longer distracted by a visual stimulus in the visual field related to the damaged colliculus.

One of the most fundamental functions of the brain is the translation of sensory input into movement output. We have studied one system within the brain, that related to vision and eye movements, to try to understand this brain function. We investigated two specific questions in man and monkey. First, how our eye movements act on our visual perception and the physiological activity underlying it. Second, how visual stimulation leads to eye movements, and what the role of the superior colliculus and frontal cortex is in this process.

On the first question, we continued to study the classical question of why rapid (saccadic) eye movements are not accompanied by a sensation of blur (see the 1976 annual report). This line of research originated in psychophysical observations by Campbell and Wurtz suggesting that the main factor in accounting for the lack of perception of saccadic blur under normally illuminated conditions is a masking effect of the clear visual scene present before and after a saccade acting on the stimuli present during the saccade. Last year we reported initial results from physiological experiments on awake behaving monkeys which investigated the possible mechanisms underlying this perceptual masking. Briefly, we found that masking stimuli carefully selected to strongly stimulate a neuron in the primary visual cortex before and after a saccade altered the neuron's response to stimuli during saccades.

We continued these physiological experiments this year using awake monkeys trained to fixate their gaze on a point of light on a screen in front of them. During the time the monkey was fixating, we found the location in the visual field of a stimulus which was effective in activating the particular cell under study (the visual receptive field of the cell). We next had the monkey make a saccadic eye movement across the visual stimulus to determine whether the cell was responsive during the eye movement. Alternatively, while the monkey fixated, we swept the stimulus rapidly across the receptive field.

We (Judge, Wurtz and Richmond) found that the presence of a masking stimulus before the saccade was sufficient to produce an elimination of the response of a neuron in visual cortex to stimulation during a saccade. But masking stimuli present after the saccade rarely produced such an effect. In this latter case the response to the stimulus present during the saccade merged into the response to the masking stimulus after the saccade so as to be indistinguishable except on the basis of the latencies of the neural response. These experiments were repeated when the monkey simply fixated a spot on the screen and the stimuli were moved rapidly across the receptive field of a cell preceded or followed by a stationary masking stimulus on the receptive field. The results were the same as when stimulus motion was produced by eye movements.

We have also shown that non-optimal "noise" stimuli (randomly patterned or unpatterned stimuli) produce masking effects similar to those of optimal

stimuli. This is critical since in the real environment stimuli present before or after saccades would rarely be optimal for any particular neuron.

To directly relate these physiological observations to human perception, we used the same stimuli used to study neurons in the monkey's brain to study perception in humans. Specifically, we determined visual detection thresholds for rapid stimulus movement when that movement was preceded or followed by a stationary masking stimulus. When a masking stimulus was presented before a rapid stimulus movement, it eliminated or reduced the perception of the stimulus movement over a time course paralleling the time course seen for neurons in the monkey visual system. This was true both for the optimal masking stimulus and the random pattern masking stimulus. What was particularly striking was that the perceptual masking effect of stimuli after a rapid stimulus movement was not detected when the response of the monkey cortical cells to the stimuli would be confused. This is a very interesting effect, since it suggests that for perceptual masking to occur it is not necessary that cortical cell responses be eliminated (as is the case when the masking stimulus precedes the rapid stimulus movement) but only that they be confused or camouflaged (as is the case when the masking stimulus follows the rapid stimulus movement).

In the other branch of the visual pathway, the superior colliculus, we find a different effect of rapid eye movements on cellular activity in the monkey's brain. As reported last year, the visual response of cells in the superficial layers of the superior colliculus show a corollary discharge associated with saccadic eye movement. That is, as the monkey moves its eyes, a corollary of the motor activity going to move the eye muscles projects to the superior colliculus and suppresses the visual activity in the colliculus resulting from the eye movement. This year we (Richmond, Wurtz and Judge) investigated whether the superior colliculus, like the visual cortex, shows a visual masking effect. It does. The response to a spot of light falling on the receptive field of a colliculus cell could be completely eliminated by a preceding masking stimulus which fell on the same part of the receptive field.

When a small spot of light was placed far outside the excitatory visual receptive field and used as a preceding masking stimulus, the response of the cell to the flashed spot in the field was markedly attenuated although usually not eliminated. The effect of the distant stimulus is roughly proportional to its distance from the edge of the excitatory receptive field in the same hemifield, but when the distant stimulus crosses the vertical meridian its effect is greatly reduced The distant stimulus did not affect the rate of discharge of the cell when tested alone.

A masking effect also modifies the response of these superior colliculus cells to stimulus movement. Many cells have only a weak response to rapidly moving visual stimuli which cross both the center and the surround of a visual receptive field. If the sweep of the visual stimulus crossed only the central excitatory visual field, the response of the superior colliculus

cells to the sweep stimulus increased. The effects on both stationary and moving stimuli by stimuli outside the excitatory receptive field area probably result from stimulation of the antagonistic surrounds of collicular receptive fields. While the effects of the antagonistic surrounds of superior colliculus cells are not surprising, our experiments demonstrate the importance of these visual interactions on the response of the cells during eye movements. These masking effects in the superior colliculus act in addition to the suppression resulting from a corollary discharge to reduce the response of colliculus cells during eye movements. The physiological activity in the superior colliculus, however, may be more closely tied to the initiation of eye movements than to visual perception.

The second direction of research which we have pursued in the last year (Albano, Healy and Wurtz) concerns the effect of lesions of the primate superior colliculus upon the initiation of eye movements. Recent studies of the role of the superior colliculus in vision and eye movements in this laboratory have utilized small lesions, involving restricted areas of visual field representation, and simple stimulus conditions, using single detection or saccade targets. The deficits resulting from these lesions were limited primarily to a slight decrease in saccade accuracy and an increase in saccade latency to a visual target within the damaged field. Even with large bilateral lesions, numerous other investigators also have found only slight or transient effects upon either the detection of visual stimuli or the generation of saccadic eye movements. Several authors have noted, however, a paucity of spontaneous eye movements and a loss of reactivity to their environment. Even animals with unilateral lesions apparently sit in their cages with their eyes deviated to the side of the lesion, while bilaterally lesioned animals "stare aimlessly into space." These observations present a puzzle: The spontaneous oculomotor behavior of the monkey is abnormal and yet in experimental tests the animal is clearly able to detect and make saccades to visual stimuli.

This year we have focused our attention upon two of these observations in experiments done in collaboration with Mort Mishkin of the Laboratory of Neuropsychology, NIMH. First, how is the spontaneous oculomotor behavior of the rhesus monkey modified by large lesions of the colliculus. And second, how is this loss of reactivity to the environment reflected in visuo-oculomotor behavior.

Using electrooculogram recordings, we first studied the pattern of eye movements in two normal monkeys with head restrained. While viewing a complex visual array, the monkeys display a diverse repertoire of large and small saccadic eye movements; the smaller eye movements of less than 15° predominate. Some of these smaller eye movements take the form of a glance where the intervening fixation may be no longer than the latency for a saccade of the same size. When in the dark, the alert, normal monkeys no longer execute the smaller eye movements (including glances) with such high frequency but the rate at which the large eye movements occur is the

same as in the light, suggesting that the smaller eye movements are visually elicited. In addition, while performing a visual fixation task, the normal monkeys were readily distracted by irrelevant and unrewarded stimulus onset and movements occurring in the peripheral visual field. This distraction was expressed by a saccadic eye movement toward the stimulus. If the stimulus was presented repeatedly or predictably the animal makes no saccades to the stimulus.

Later, one monkey received a large bilateral lesion to the superior colliculus made under visual inspection and the other monkey received a unilateral lesion. After one week of recovery and throughout the several weeks following surgery we found a selective reduction in the frequency of the smaller saccades and glances made to the damaged visual field, but activity in the dark (which does not include the smaller saccades) remained largely the same. In addition, these monkeys were no longer distracted by the onset and movement of a stimulus in their damaged field although the unilaterally lesioned monkey continued to be distracted by a stimulus in the visual field contralateral to the remaining superior colliculus. These effects are not a result of simple sensory or motor loss since these animals could detect and make saccades to visual stimuli within the central visual field.

Both of these experiments show that loss of the superior colliculus in the monkey leads to loss of unrewarded , visually initiated saccadic eye movements. The pattern of this loss is complex, however, and the experiments leave open the question of whether the "lost" saccades represent visual "scanning", "search" or "exploratory" movements. These results do not, however, support the traditional notions of the colliculus as a visual-eye movement reflex center but are consistent with a more general role in visual attention and a shift in gaze.

Experiments also concerned with the initiation of saccadic eye movements concentrated on cells in the prefrontal cortex of the monkey which discharge before saccadic eye movements (Judge and Richmond). It has been known for some time that there are no such neurons in the frontal eye fields, but it was unclear whether there might be such neurons in the middle part of the principal sulcus, anterior to the frontal eye fields. Our interest in this particular area was that it had been recently shown to project to the superior colliculus, where there are neurons which fire before eye movement, and we wished to test the possibility that these neurons are driven from prefrontal cortex. We therefore searched for cells that discharge before saccadic eye movements. The results of experiments were negative; no neurons were found which clearly fired before eye movements which were not visual sensory neurons. A very intriguing discovery, however, was that a sub-set of neurons in the mid-principalis region have powerful short-latency auditory responses. These neurons seem to be best excited by click stimuli and in several cases were conspicuously sensitive to the direction of the sounds in space.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 01031-10 LNC

PERIOD COVERED

October 1, 1977 through September 30, 1978

TITLE OF PROJECT (80 characters or less)

The Conversion of Phenylalanine to Tyrosine

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.5	3.5	1

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☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A new procedure has been developed that permits the early diagnosis of hyperphenylalaninemia due to a deficiency of pterin cofactor. The test does not involve surgery since it is carried out on blood samples rather than on liver.

The in vivo activity of hepatic phenylalanine hydroxylase has been shown to be under hormonal control.

Project Description:

The objective of this research project is the detailed description of the complex enzyme system that catalyzes the conversion of phenylalanine to tyrosine. The specific goal is the analysis of the structure, mechanism of action, and modes of physiological regulation of the essential components of the hydroxylation system. These components include phenylalanine hydroxylase dihydropteridine reductase, and tetrahydrobiopterin.

A related objective is to use this basic knowledge of the phenylalanine hydroxylase system to increase our understanding of the disease, phenylketonuria, (PKU), as well as its variants. Specifically, the goal is to devise methods of detection and therapy for these diseases, as well as to understand their pathogenesis.

We have continued to try to develop methods that would facilitate the early detection of the two new variant forms of PKU, that caused by a deficiency of dihydropteridine reductase as well as that caused by a deficiency of biopterin. Until now, the only rapid, reliable tests for these diseases have been based on determination of reductase and biopterin in liver biopsy samples.

During the last year, we have succeeded in developing a test that can detect at a very early age hyperphenylalaninemia (HPA) due to a deficiency of biopterin. The test is based on the observation that high levels of phenylalanine in blood lead to a prompt increase in the blood levels of biopterin. We have shown that phenylalanine elicits this response in both normals and in patients with classical PKU. In contrast, the patient with a hepatic deficiency of biopterin does not show this response to a phenylalanine challenge. These results strongly support the following two conclusions: a) this patient is blocked in the biosynthesis of biopterin; b) biopterin biosynthesis is normally regulated by phenylalanine. The diagnostic procedure consists of the administration to the patient of a load of phenylalanine (100 mg/kg). Phenylalanine and biopterin are then determined in blood samples taken at hourly intervals.

We have continued our studies on the activation of phenylalanine hydroxylase by phosphorylation. We have shown that the two major forms of the enzyme, which are separated on calcium phosphate columns, differ in their phosphate contents, one containing about 0.25 moles of P per enzyme subunit (molecular weight of the subunit, 50,000) and the other containing 0.5 mole of P per subunit. On phosphorylation *in vitro*, each of these forms is converted to a new form which is capable of being separated from the others on the calcium phosphate column. Phosphorylation is accompanied by activation of the enzyme. Since it is known that the hormone, glucagon, is capable of increasing the activity of adenylate cyclase in liver, and that this enzyme is indirectly involved in many protein phosphorylations, we expected that glucagon might be able to activate phenylalanine hydroxylase in rats. We have shown that this occurs and that the

activation involves phosphorylation. This is the first demonstration that hepatic phenylalanine hydroxylase activity is under hormonal control.

Publications:

Jedlicki, E., Kaufman, S., and Milstien, S.: Partial purification and characterization of rat liver phenylalanine hydroxylase phosphatase. J. Biol. Chem. 252: 7711-7714, 1977.

Donlon, J., and Kaufman, S.: Modification of the multiple forms of rat hepatic phenylalanine hydroxylase by in vitro phosphorylation. Biochem. Biophys. Res. Commun. 78: 1011-1017, 1977.

Kaufman, S.: Mixed function oxygenases -- General considerations. In Usdin, E., Weiner, N., and Youdim, M.B.H. (Eds.): Structure and Function of Monoamine Enzymes. New York, Marcel Dekker, Inc., 1977, pp. 3-22.

Butler, I.J., Koskow, S.H., Krumholz, A., Holtzman, N.A., and Kaufman, S.: A disorder of biogenic amines in dihydropteridine reductase deficiency. Ann. Neurol. 3: 224-230, 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 01032-10 LNC

PERIOD COVERED

October 1, 1977 through September 30, 1978

TITLE OF PROJECT (80 characters or less)

Biosynthesis of Catecholamines

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Seymour Kaufman	Chief, Lab. of Neurochem.	LNC NIMH
OTHER:	Robert Pollock	SeniorStaff Fellow	LNC NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Neurochemistry

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2	1.5	.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The objective of this work is the detailed description of the hydroxylation reactions that are involved in the biosynthesis of the neurotransmitters, dopamine and norepinephrine. Recently our emphasis has been on the purification of tyrosine hydroxylase from brain and elucidation of the mechanism for its activation by protein phosphorylation.

Project Description:

Tyrosine hydroxylase catalyzes the rate-limiting step in the reaction sequence leading from tyrosine to dopamine and norepinephrine. Factors which change the activity of tyrosine hydroxylase can therefore readily change the levels of dopamine and norepinephrine. One of these factors is thought to be an activation of tyrosine hydroxylase by protein phosphorylation.

When tyrosine hydroxylase is incubated with protein kinase, ATP and cAMP, the kinetic properties of tyrosine hydroxylase are altered. The Km for the tetrahydropterin cofactor is decreased; the Ki for catecholamine is increased; and the pH optimum is shifted to higher pH. These changes would result in an increase in tyrosine hydroxylase activity under physiological conditions in which the tetrahydropterin is not saturating, catecholamines are present, and the pH near neutrality.

Efforts were made to purify tyrosine hydroxylase in order to facilitate the study of the mechanism for its activation. A purification scheme has been developed that allows a thirty-fold purification of tyrosine hydroxylase from the bovine caudate nucleus with a recovery of thirty percent of the original activity. It can be estimated that this enzyme preparation is about fifteen percent pure. A major effort is being made to further purify tyrosine hydroxylase to homogeneity.

We have used the partially purified tyrosine hydroxylase to examine several models for the activation of the enzyme by protein phosphorylating conditions. Previous work in this lab had used antibody to tyrosine hydroxylase to look for direct phosphorylation of the enzyme by [γ-^{32}P]ATP but no ^{32}P incorporation could be detected. This result indicates that tyrosine hydroxylase is not directly phosphorylated to form an activated enzyme that retains the phosphate. Another model is that tyrosine hydroxylase is itself phosphorylated but the resulting activated enzyme releases the phosphate. To test this model, we sought the formation of ^{32}Pi during the activation of tyrosine hydroxylase by [γ-^{32}P]ATP. The results indicate that phosphate is not released during activation but the detection limits of the test method must be lowered in order to definitely rule out this model.

Models for the activation of tyrosine hydroxylase by phosphorylating conditions have been considered in which another protein is the one directly phosphorylated. One such model is that another protein is phosphorylated and then this protein binds stoichiometrically to tyrosine hydroxylase to activate it. To test this model, tyrosine hydroxylase was incubated with [γ-^{32}P]ATP during activation then purified by sucrose density gradient centrifugation. Tyrosine hydroxylase remained activated during this purification but was not associated with the ^{32}P label. Thus, this model is apparently incorrect. Another model is that a contaminating enzyme is activated by phosphorylation and this enzyme then modifies tyrosine hydroxylase to activate it. Results from the sucrose density gradient centrifugation

indicate that the native and activated tyrosine hydroxylases are similar in sedimentation behavior. More subtle modifications of tyrosine hydroxylase by phosphorylating conditions have not yet been examined.

Publications:

Diliberto, E.J. and Kaufman, S.: Studies on the mechanism of catechol oxidation catalyzed by dopamine-β-hydroxylase. Fed. Proc. 37: 1421, 1978.

Pollock, R.J. and Kaufman, S.: Dihydrofolate reductase is present in brain. J. Neurochem. 30: 253-256, 1978.

Hoeldtke, R., and Kaufman, S.: An in vivo tritium release assay of dopamine-beta-hydroxylase activity in sympathetic neurons. Biochem. Pharmacol., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 01034-10 LNC

PERIOD COVERED

October 1, 1977 through September 30, 1978

TITLE OF PROJECT (80 characters or less)

The Biochemical Basis of Skeletal Muscle Hypertrophy

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Seymour Kaufman	Chief, Lab. of Neurochem.	LNC NIMH
OTHER:	Philip Fleckman	Medical Officer (Res.)	LNC NIMH
	Herman Vandenburgh	Staff Fellow	LNC NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Neurochemistry

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INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	2	.5

CHECK APPROPRIATE BOX(ES)

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The object of this project is the elucidation of the biochemical mechanism of compensatory skeletal muscle hypertrophy. These studies will give greater insight into the physiological regulation of normal and pathologic skeletal muscle metabolism and into the role of the nervous system in this regulation. Present topics of investigation include: 1) The relationship between compensatory skeletal muscle hypertrophy and endurance training; 2) the relationship between skeletal muscle and connective tissue in hypertrophy; the relationship between stretch-induced hypertrophy and muscle protein synthesis and degradation; 4) localization of phosphoinositide changes in hypertrophy; and 5) cyclic nucleotide changes in hypertrophy.

HS-6040
Rev. 10-76)

Project Description:

The object of this project is the elucidation of the biochemical mechanism of compensatory skeletal muscle hypertrophy. The animal model used in this project is the hypertrophy of the soleus and plantaris muscles which occurs when the synergistic gastrocnemius muscle is incapacitated by tenotomy of the achilles tendon. The contralateral sham-operated leg serves as control. The in vitro model employs mechanically stretched monolayers of embryonic chicken myotubes.

This laboratory demonstrated in earlier work that compensatory skeletal muscle hypertrophy is accompanied by connective tissue cell proliferation and that the enhanced RNA synthesis associated with hypertrophy is localized to these proliferating non-myofiber elements. The proliferation is not essential for hypertrophy since DNA synthesis can be almost completely inhibited without significantly affecting muscle hypertrophy. Also, collagen content is the same in hypertrophied and control muscle. These findings suggest that increased connective tissue metabolism is not essential for the initiation of muscle hypertrophy. We examined specific myofibrillar protein changes in hypertrophying muscles in which DNA synthesis is inhibited, and they do not differ from those in normal hypertrophied muscle. In using these procedures, a new and more specific criterion for skeletal muscle hypertrophy, i.e., increase in myofibrillar protein, is evolving.

Other investigators have hypothesized that compensatory skeletal muscle hypertrophy is due to stretching of the hypertrophying muscle, which leads to increased tension and increased work. Investigation of the relationship between stretch and myofibrillar protein synthesis and degradation are in progress using a technique developed in this laboratory of stretching monolayers of chick embryonic skeletal myotubes in vitro. The use of both an in vivo and an in vitro model to study skeletal muscle hypertrophy offers a great advantage over the use of either system alone. With the in vitro model we have shown that the changes which occur in vivo also occur in vitro, e.g., increased amino acid accumulation, increased incorporation of amino acids into total cellular protein and muscle specific protein (myosin heavy chain) and increased accumulation of total cellular protein and muscle specific protein. The in vitro system has the advantage that one can prepare pure muscle cultures, devoid of connective tissue cells. We are currently investigating which of the above changes occur specifically in the muscle cells.

We have shown increased incorporation of myoinositol into inositol phospholipid early in muscle hypertrophy. A radioautographic technique has been developed to localize this inositol effect. Preliminary data indicate that, unlike the increase in RNA synthesis, the increase in inositol incorporation takes place in the muscle cells and may therefore mark an early event in the biochemical changes of the hypertrophying muscle. We are presently investigating this "inositol effect" in both the in vitro and in vivo models for hypertrophy. Because an increase in guanosine

3',5'-cyclic phosphoric acid (cyclic GMP) accompanies the "inositol effect" in many other stimulated tissues, we are examining the changes in cyclic GMP in hypertrophying muscle.

As we study biochemical events that occur earlier in hypertrophy, and in systems in which the hypertrophy is produced by different means, we hope to gain insight into the biochemical mechanism of skeletal muscle hypertrophy.

Publications:

Fleckman, P., Bailyn, R.S., and Kaufman, S.: Effects of the inhibition of DNA synthesis on hypertrophying skeletal muscle. J. Biol. Chem. 253: 3320-3327, 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 01035-10 LNC

PERIOD COVERED

October 1, 1977 through September 30, 1978

TITLE OF PROJECT (80 characters or less)

The Process of Lysogeny

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Howard Nash	Medical Officer (Research)	LNC NIMH
OTHER:	Yoshiko Kikuchi	Visiting Associate	LNC NIMH
	Thomas Pollock	Guest Worker	LNC NIMH
	Carol Robertson	Biologist	LNC NIMH

COOPERATING UNITS (if any) Laboratory of Molecular Genetics, NICHD; Laboratory of Molecular Biology, NCI; Laboratory of Molecular Biology, NIAMDD; Division of Cancer Biology, Frederick Cancer Research Center; Department of Microbiology, Univ. of Michigan Med. Sch., Dept. of Microbiology, Brown University.

LAB/BRANCH

Laboratory of Neurochemistry

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4	3	1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The biochemistry of the integration of the DNA of a virus into the chromosome of its bacterial host has been studied. The proteins that carry out this naturally-occurring genetic recombination have been purified and characterized. When acting alone, each protein component is without permanent effect on DNA; recombination is produced only by the combined action of all the components. The component encoded by the bacterial host consists of at least two proteins, each of which is of low molecular weight and is associated with the bacterial chromosome. These proteins include elements responsible for the specific use of supertwisted DNA in integrative recombination. The component encoded by the virus is a protein that functions as a DNA sequence specific element for recombination. The DNA sequences on the viral chromosome that are involved in the specific recognition of this protein have been mapped.

Using the techniques developed in the study of integrative recombination, a second naturally-occurring site-specific recombination has been achieved in cell-free extracts.

PHS-6040
(Rev. 10-76)

Project Description:

All living systems vary the organization and content of their genome through a process called genetic recombination. We have been studying the biochemistry of a particular genetic recombination that is normally responsible for the insertion of the DNA of a virus into the chromosome of its bacterial host. These studies have been enabled by our development of a cell-free system that carries out this recombination essentially as it occurs in nature. We have used this system to characterize the DNA substrate and products of the reaction and to define the enzymes and cofactors that carry out the breakage, realignment, and rejoining of the DNA double helix during a genetic recombination.

Our previous studies have shown that integrative recombination results from the interaction of a supertwisted substrate DNA with proteins encoded by both the bacterial host and the virus. The bacterial proteins are expressed even in cells never exposed to virus and therefore probably play a role in additional cellular processes. We have achieved an initial purification of these bacterial proteins. At least two components have been separated and some properties of the partially purified material have been determined. Our most significant findings to date show that: 1) the proteins are of low molecular weight; 2) they associate with one another; 3) at least one of these proteins interacts preferentially with supercoiled DNA; and 4) they are naturally found as part of the nucleoid of E. coli.

In contrast to the relatively primitive studies of the bacterial component our understanding and control of Int protein, the product of the viral int gene, is very advanced. Using a specially constructed strain that over-produces Int, we have obtained milligram quantities of nearly homogeneous protein. We have confirmed that, in the absence of the suitable bacterial proteins, Int can neither carry out recombination nor otherwise covalently alter DNA. Three new approaches to the understanding of the role of Int in recombination have been made possible by the availability of large amounts of pure protein. First, we have completed our studies of the ability of DNA and Int to form complexes that are retained on membrane filters. These studies have shown that Int binds well only to DNA that contains the viral attachment site, the place where the recombining DNA is broken and rejoined. In addition, studies of Int binding to variants of the attachment site with known alterations in DNA sequence show that all the elements on DNA that are required for a strong interaction with Int cluster to one side of the attachment site region. Second, we have shown that when Int complexes with DNA, a specific region of the DNA is protected from attack by exogenous deoxyribonucleases. The protected region is 30-50 base pairs long, indicating that Int protein interacts with several turns of the double helix. In collaboration with others, we will attempt to align this protected fragment with respect to the nucleotide sequence map of the attachment site region. Third, in collaboration with Dr. R. Yuan, complexes between Int and DNA have been studied by electron microscopy. As expected, these are seen to be specifically located

at the attachment site. In agreement with the size of the protected fragment, the protein portion of the complex appears to be quite large, indicating that aggregates of 8-10 monomers of Int form on a single DNA site. Taken together, our studies show that Int is a specificity element, directing the recombination to attachment sites. Our continuing studies will investigate how this element combines with the bacterial proteins to yield site-specific recombination.

We are extending our studies of integrative recombination with purified proteins to other site-specific recombinations. Recently, we have demonstrated int-promoted recombination between two identical prophage attachment sites. We can now compare this reaction to integrative recombination and determine the biochemical origin of the differences in the physiology of these two naturally-occurring recombinations.

Publications:

Nash, H.A., Mizuuchi, K., Weisberg, R.A., Kikuchi, Y. and Gellert, M.: Integrative recombination of bacteriophage λ - The biochemical approach to DNA insertions. In Shapiro, J., Bukhari, A. and Adhya, S. (Eds.): Plasmids, DNA Insertion Elements, and Episomes. New York, Cold Spring Harbor Laboratory, 1977, pp. 363-373.

Williams, J.G.K., Wulff, D.L. and Nash, H.A.: A mutant of Escherichia coli deficient in a host function required for phage lambda integration and excision. In Shapiro, J., Bukhari, A. and Adhya, S. (Eds.): Plasmids, DNA Insertion Elements, and Episomes. New York, Cold Spring Harbor Laboratory, 1977, pp. 357-361.

Court, D., Adhya, S., Nash, H. and Enquist, L.: The phage λ integration protein (Int) is subject to control by the cII and cIII gene products. In Shapiro, J., Bukhari, A. and Adhya, S. (Eds.): Plasmids, DNA Insertion Elements and Episomes. New York, Cold Spring Harbor Laboratory, 1977, pp. 389-394.

Nash, H.A.: Integration and excision of bacteriophage λ. Current Topics in Microbiology & Immunology 78: 171-199, 1977.

Nash, H.A., Kikuchi, Y., Mizuuchi, K., and Gellert, M.: Integrative recombination of bacteriophage lambda: Genetics and biochemistry. In Hofschneider, P.-H. and Starlinger, P. (Eds.): Proceedings of the 28. Mosbacher Colloquium. Berlin, Heidelberg, Springer-Verlag, 1978, pp. 21-27.

Nash, H.A., Enquist, L.W. and Weisberg, R.A.: On the role of the bacteriophage λ int gene product in site specific recombination. J. Mol. Biol. 116: 627-631, 1977.

Mizuuchi, K., Gellert, M., and Nash, A.: Involvement of supertwisted DNA in integrative recombination of bacteriophage lambda. J. Mol. Biol., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 01036-06 LNC

PERIOD COVERED
October 1, 1977 through September 30, 1978

TITLE OF PROJECT (80 characters or less)
Heparin-Induced Conformations: Model for Intracellular Polysaccharide-Protein Interactions

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Audrey L. Stone Research Chemist LNC NIMH

COOPERATING UNITS (if any)
None

LAB/BRANCH
Laboratory of Neurochemistry

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.1	0.5	0.6

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES XX (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords) Physicochemical forces which sequester cell solutes from soluble form to organized structures are investigated with regard to molecular conformations of components and interacting species. Earlier results showed that ordered, negative charges (glycosaminoglycans) have specific ordering effects on amine cations. The current project studies polyanions inducing conformational constraints in otherwise nonordered cationic polypeptides. Polysaccharides (PS) examined for conformation-promoting-effects on poly-L-lysine showed little specificity; all with sufficient anionic density promoted a high degree of α-helical conformation; specificity dependent upon peptide structure revealed that interactions of heparin (and other polyanions) with copolymer L-lysine:L-tyrosine promote the β-protein structure, while those with copolymer L-lysine:L-phenylalanine induce α-helical order. That the nature of conformational changes in protein models due to interaction with heparin depends upon constellations of amino acids and their proclivity for α-helix or β-structures is significant in the molecular basis of multianion action in granule formation and enzyme activation. Currently, heparin activation of tyrosine hydroxylase and heparin isoelectric focusing have been studied on the basis of molecular characterization.

Project Description:

Anionic polysaccharides (glycosaminoglycans (GAG)) are intimately involved in the biological formation and function of inter- and intra-cellular matrices and some, such as heparin, are known to stimulate or inhibit certain enzyme systems. Intracellular storage regions, or discrete granules, occur in various organs of many species, including synaptic processes of catecholaminergic neurons. Physicochemical forces which sequester cell solutes from a soluble form to a relatively insoluble, organized structure can be investigated from the standpoint of the molecular conformations of the components and of their interacting species. Investigation of the macromolecular structure of anionic polysaccharides in solution was undertaken at the onset of this project in the early 60's with the subsequent elucidation of helical conformation in heparin and other GAG in solution.

The histology, chemical composition, and explosive release of the histamine containing granule of the mast cell were well described at that time; therefore, the heparin-histamine system was used as a conceptual model for storage and release of small potent biologically active molecules (although other multi-anion/cations apply in different systems). Thus, the conformational aspects of heparin/histamine (or cationic aromatic dyes) binding were studied using optical methods of absorption and circular dichroism (CD). The results of those early studies were the first to demonstrate ultraviolet asymmetric absorption bands for heparin and other GAG. Results with the cationic amine dyes led to the first reports of helical structures for these polysaccharides in solution. Both methods showed that GAG comprise two conformational classes in solutions near pH 7. Specific conformations were elucidated for related plant polysaccharides. Direct X-ray analysis recently performed in other laboratories now confirm the various helical patterns predicted by the early reports (M-NC-21 1964-67). The results also showed that ordered negative charges have profound and specific ordering effects on amine cations.

The current project extends these basic molecular findings to the study of a third component of inter- and intra-cellular matrices, e.g., the polypeptide. As with small amines, acid polysaccharides (PS) play a role in inducing conformational constraints in cationic polypeptides. The major objective of the current project is to quantitatively determine the conformation-altering effect of various anionic PS, and to determine possible specificity of this effect on the basis of PS conformations revealed earlier in this project. These model interactions will be applied directly to studies of enzyme activation and sequestration by heparin, and related biopolymers, in simulated matrix conditions.

The methods are measurements of molecular asymmetries by extrinsic and intrinsic Cotton effects using the Cary Model 6001 circular dichroism apparatus. The metachromatic reactions developed earlier in the project (measured spectrophotometrically and by circular dichroism) provide a quantitative measure of the degree of interactions with anionic sites. Computer models for determination of the proportion of α-helical, β- or non-ordered structure in the polypeptide reveal the degree and kind of conformation induced by a given PS.

Studies of striatal tyrosine hydroxylase utilize dissection of mouse striatum and partial purification by means of a one-step separation by affinity chromatography.

Results using purified PS from plant, bacterial and mammalian sources, well-characterized as to the chemical nature of the anionic groups and the charge density, showed (Z01 MH 01036-04 LNC) that the non-ordered form of PLL was altered markedly by a strong electrostatic interaction with polysulfates, polyuronates, and mixed polyanionic groups on a homo- or complex polysaccharide backbone. Furthermore, there was little specificity in the interactions since both classes of GAG and all PS promoted a high degree of α-helical conformation in PLL. Subsequent studies revealed that mixed polypeptides show specificity of interaction with PS depending upon the nature of the non-basic amino acid residues. Heparin induces α-helix in L-lysine copolymer with L-phenylalanine (PLP) while copolymer L-lysine:L-tyrosine (PLT) forms the β-protein structure. Metachromatic measurements showed that the stoichiometry of the reaction was 1-to-1 with positive lysyl residues and negative heparin charges for the poly(L-lysine:L-tyrosine) and close to 1-to-1 with the poly(L-lysine:L-phenylalanine). The specificity of the biological functions of heparin and other GAG, therefore, may emerge to be due to the capacity to alter the conformations of specific amino acid sequences in a given protein, rather than to solely electrostatic interactions. Methods for looking at the stoichiometry and interactions of certain specialized heparan sulfates with PLL, PLT, and PLP have been developed and indicate that this glycosaminoglycan also interacts strongly with lysine containing polypeptides.

Affinity chromatographic technique for a single passage of striatal extract using heparin fixed to Sepharose 4B showed that a low degree of substitution (<1 mg/ml) was inadequate to obtain significant increases in specific activity of tyrosine hydroxylase. Methods were developed which showed that heparin fixed to the Sepharose 4B bead was available as a polyanion and could be measured by interaction with methylene blue under our conditions. Furthermore, a 3 to 4 fold increase in the amount of heparin substituted on activated Sepharose 4B was achieved by subsequent modification of the reaction conditions.

The significance of molecular conformations of organized multianions (such as the glycosaminoglycans) in brain function and mental health was not apparent to most ten years ago. However, it was clear then that the structure of inter- and intra-cellular matrices through which neurotransmitters pass, must play a role. The early results demonstrated that small cations were held in specific and highly ordered fashion by various polyanions, described a third parameter of the metachromatic reaction, and set down general techniques for measuring the above kinds of interaction. The findings with cationic polypeptides that such complexes can regulate polypeptide conformation, depending upon the nature of the amino acids neighboring cationic residues, may have direct bearing on the biological aspects of amine storage in the CNS.

Another visible role for basic studies of molecular conformations is seen, for example, in the activation of certain enzymes by heparin. Tyrosine hydroxylase, the enzyme responsible for DOPA formation, is known to occur in both soluble and insoluble forms, of which the former is markedly stimulated by heparin. The significance of our studies on the conformational aspects of heparin-polypeptide interactions lies in the quantitative measures of the stoichiometry, which permit the proposal of a molecular model to explain, for example, the activation of tyrosine hydroxylase, and a possible mode of regulation of this enzyme, natural as well as pharmacological. Our studies define a rational approach to selecting agents which might affect this enzyme system in a predicted manner.

During the past four months I have studied the regulation of striatal tyrosine hydroxylase by heparin-like glycosaminoglycans of the CNS, reexploring the molecular characteristics and specificity of the interaction. Partially purified mouse striatal tyrosine hydroxylase, (up to 10 fold purified in a one-step heparin affinity column) was used as the source of the enzyme; heparin fractions, heparan sulfate and other polyanions were looked at in regard to their conformational alterations of polypeptide models. Three new findings of importance emerged, 1) one of our heparin fractions, separated by mass, is significantly more potent in activating tyrosine hydroxylase on an equivalent charge basis than the bulk preparation; low molecular weight fraction (<6000) and high molecular weight fraction (>28,000) are not active at the same equivalent charge; 2) heparan sulfate does not activate tyrosine hydroxylase despite its heparin-like components; and 3) a potent inhibitor of the enzyme was developed; this provides a one column-step purification of 5-10 fold when coupled to Sepharose B, similar to that of the heparin substituted affinity gel.

The in vitro inhibition and stimulation appear to be interrelated, since the markedly inhibited enzyme can be converted to the fully activated enzyme by heparin (possibly by specific interactions with active enzyme or by protection in the in vitro assay), and conversely, at higher inhibitor concentrations, activation by heparin can be prevented. These results bear on possible regulatory properties of tyrosine hydroxylase in vivo.

Last summer in collaboration with a Visiting Scientist, Dr. P. Righetti, we studied the mechanism of the anomalous isoelectric focusing of heparin using our metachromatic methods for measuring heparin-amine interactions. We showed that heparin formed complexes with the carrier ampholytes which varied in stoichiometry with pH, heparin concentration, and heparin-to-ampholyte ratio, regardless of molecular weight (except in the two extremes of the range). Thus, heparin molecules were immobilized by binding to carrier ampholytes in the various pH zones, and a general mechanism of banding of various polyanions upon isoelectric focusing was elucidated.

The proposed course of the project is a continuation of the basic studies of glycosaminoglycan-polypeptide interactions and studies on the regulation of striatal tyrosine hydroxylase by heparin-like glycosaminoglycans of the CNS. Collaboration with Dr. Robert Rosenberg on the optical properties

of his "highly-active heparin (antithrombin-binding heparin)" is anticipated. Further quantitation of the tyrosine hydroxylase active fraction will be attempted, and if obtained in adequate quantity affinity chromatography procedures can be modified to yield higher purification with a single column step, as well as studies on the effect of this fraction on the Km for biopterin. The techniques of conformational potential in polypeptides using heparin interaction have been applied to myelin basic protein in collaboration with Dr. Marian Kies, and this will be continued. Unless we would be able to obtain a spectropolarimeter in our laboratory, further experiments will be conducted in other laboratories at NIH due to 1) an arbitrary change in accessibility of the NIMH spectropolarimeter preventing proper overseeing in the care and maintenance of this instrument for serious scientific use, and 2) deterioration of the optics of this instrument. It is suggested that the Laboratory of Neurochemistry be given the charge of revamping and housing this expensive optical system so that it might be used to a greater extent.

Publications:

Righetti, Pier Giorgio, Brown, Ronald P. and Stone, Audrey L.: Aggregation of carrier ampholytes on heparin and other acidic polysaccharides in isoelectric focusing. Biochim. Biophys. Acta, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 01037-10 LNC

PERIOD COVERED

October 1, 1977 through September 30, 1978

TITLE OF PROJECT (80 characters or less)

The Role of the Cell Membrane in Cellular Organization - A Molecular Study

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
5.0	4.0	1.0

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☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The general aim of this project is to determine the chemical interactions which occur at the surfaces of cells which affect cellular differentiation and organization. Specifically we have studied one type of interaction, plasma membrane receptor mediated entry of proteins into the cell cytosol. These studies have been done by developing techniques to construct artificial protein hybrids containing the active fragment of diphtheria toxin and another receptor specific binding protein. Such artificial protein hybrids may have value as a new class of pharmacologic reagents.

HS-6040
(Rev. 10-76)

Project Description:

The general aim of this project is to determine the chemical interactions which occur at the surfaces of cells which affect cellular differentiation and organization. The major specific aim of the program is to synthesize a new class of pharmacologic reagents which will permit the modulation of specific cell types in a manner unobtainable with conventional reagents. This is being accomplished by the synthesis of artificial protein hybrids which utilize the process of receptor-mediated protein transport.

Receptor-mediated protein transport is the process by which cells bind an extracellular protein to a specific cell surface receptor and then transport this protein across the cell membrane to the cell interior. This process is utilized in nature to direct the entry of certain hormones into the cell interior. Examples are lactogenic hormone transport from serum to milk across mammory alveolar cells, and the transport of nerve growth factor from the adrenergic terminals to the spinal sympathetic ganglions.

Receptor-mediated protein transport has also been utilized by a number of human pathogens to direct the entry of potent enzymes to the host cell interior. Examples are C. diphtheriae, V. comma, and probably C. tetani. The toxic enzymes which gain entry are fragments of the exotoxins made by these organisms. Only one to ten molecules of exotoxin enzyme per cell are required to profoundly alter cell physiology.

Because toxin transport is receptor-mediated, a certain degree of cell type specificity is present. Intact cells lacking the receptor (mouse and rat for diphtheria toxin) are not susceptible to the toxin. All vertebrate cells, however, are susceptible to diphtheria toxin if their membranes are broken permitting toxin to gain entry to the cell cytoplasm. These toxins are therefore bifunctional molecules. They exhibit an entry function (binding to the cell receptor) and an intracellular toxin enzymatic function. These functions reside on separate parts of the toxin molecule, and chemical separation of the binding chain (B chain) and the enzymatically active chain (A chain) has been achieved.

It is our purpose to alter the cellular specificity of these toxins by replacing the natural binding chain of the toxin with a new binding chain which will bind and mediate entry to only certain selected human cell types. Such a reagent would exhibit a high degree of cell type specificity determined by the binding chain selected. We call these reagents artificial protein hybrids.

In order to achieve the above purpose the following conditions must be met:

1. Methods must be developed to synthesize artificial protein hybrids.

2. Hybrids must retain the activities of their separate binding chains and enzymatic chains.

3. The new binding chain must utilize a receptor capable of mediating protein transport to the cell cytosol.

4. The enzymatic chain and its link to the binding chain must be protected against degradation during its passage through the cell membrane.

Steps 1 and 2 have been accomplished in my laboratory during the past three years. So far, our hybrids do not exhibit toxic action towards their newly directed cell types. Methods have been developed to explore 3 and 4. At the present time our difficulty appears to be in meeting the conditions listed under 4.

CLINICAL APPLICATIONS

Bifunctional protein hybrid reagents would have application in all fields of medicine. The simplest application is to the cancer chemotherapy field since here one desires to kill one cell type without affecting other cell types. In the mental health field one wishes to modulate the activity of one cell type, a more subtle undertaking. The clinical applications listed below would use two types of active chains, diphtheria toxin fragment A which inhibits protein synthesis and cholera toxin fragment A which stimulates adenylate cyclase activity. In the following examples it is assumed that conditions 3 and 4 can be met.

Cancer Chemotherapy - Many human breast tumors including metastatic breast tumors carry lactogenic hormone receptors. A hybrid made of a lactogenic hormone and diphtheria toxin fragment A when given systemically should stop protein synthesis in all cells carrying the receptor and lead to tumor cell death. Distant metastatic lesions would also be susceptible. Destruction of at least one cell type of the adrenal glands would also occur as a side effect since the lactogenic receptors are also present at this site in high concentration.

Many malignant tumors carry unique cell surface antigens which can function as receptors such as the CEA antigen found generally in digestive tract carcinomas. A hybrid consisting of an antibody directed against CEA and diphtheria toxin fragment A should inhibit protein synthesis in these tumors and any metastatic tumors.

Mental Health Field - A hybrid of enkephalin or any of the terminal fragments of β lipotropin which bind to morphine receptors and diphtheria toxin fragment A would decrease or stop (depending on the dosage) protein synthesis in all cells carrying the morphine receptor. I believe that the effects of such a reagent are totally unpredictable.

Regional modification of brain cyclic AMP could be achieved in the following way. It is likely that different regions of the brain carry a receptor unique to that region. If a binding protein unique for the caudate was hybridized with cholera toxin fragment A, cyclic AMP levels would be stimulated in only the caudate region. Such a reagent would have much different effects than dopamine, which stimulates cyclic AMP in all parts of the brain which carry the dopamine receptor and adenylate cyclase.

Whether reagents of this sort would have benefits in treating affective disorders can not be predicted. What is certain is that these reagents would have vastly different effects than the pharmacologic reagents presently available.

Publications:

Neville, D.M., Jr. and Chang, T.-M.: Receptor-Mediated Protein Transport into Cells. Entry Mechanisms for Toxins, Hormones, Antibodies, Viruses, Lysosomal Hydrolases, Asialoglycoproteins and Carrier Proteins. In Kleinzeller, A. and Bronner, F. (Eds.): Current Topics in Membranes and Transport. New York, Academic Press, 1978, pp. 65-150.

Chang, T.-M. and Neville, D.M., Jr.: Demonstration of diphtheria toxin receptors on the surface membranes from both toxin sensitive and toxin resistant species. J. Biol. Chem. 253, in press.

Roth, J., Kahn, C.R., De Meyts, P., Gorden, P. and Neville, D.M., Jr.: Receptors for Insulin and Other Peptide Hormones in Disease States. In Bajaj, J.S. (Ed.): Insulin and Metabolism. Amsterdam, New York, Excerpta Medica - Elsevier/North-Holland, 1977, pp. 73-79.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01038-07 LNC

PERIOD COVERED

October 1, 1977 through September 30, 1978

TITLE OF PROJECT (80 characters or less)

Development of Mutant Mice for Phenylketonuria and Dihydropteridine Reductase Insufficiency

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.6	0.5	0.1

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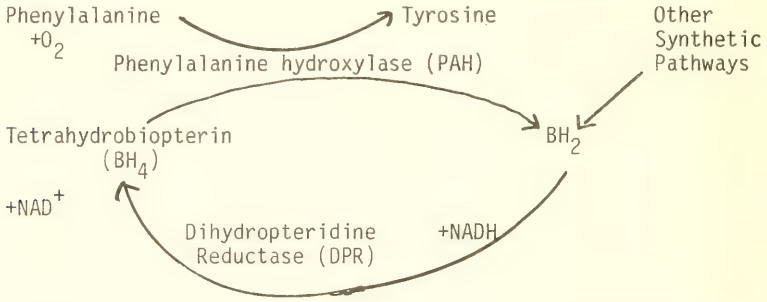
☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords) The project is a small program to develop a murine model for PKU and/or related diseases. Another objective is the elucidation of regulatory mechanisms in the expression of phenylalanine hydroxylation (PH). Criteria for a putative deficiency in PH were established: plasma phenylalanine levels, plasma phenylalanine-to-tyrosine (P/T) ratio, and hepatic levels of dihydropteridine reductase (DPR). Early results indicating that hepatic DPR in mice was not in great excess were contrary to generally accepted hypotheses. DPR insufficiency was expressed by mild symptoms of deficiency in PH. These findings pointed out inadequacies of standard programs for detecting heterozygote mouse PKU mutants. Murine DPR insufficiency was studied using mildly elevated P/T as a criterion. Putative mutant families were developed, but infertility and/or selective survival led to a loss of viable abnormal offspring at F_4 or higher. Hepatic enzyme and cofactor patterns differed among the families, reflecting the complex nature of PH. Current work elucidates the PH system in mice, shows the relationship of in vivo elevated P/T to in vitro measurable hepatic phenylalanine hydroxylase (PAH) using parachlorophenylalanine to inhibit PAH in vivo, and assesses the possibility of screening mice according to susceptibility to m-fluorophenylalanine.

Project Description:

The lack of appropriate animal models for the study of enzyme-deficient genetic diseases associated with mental retardation has checked the search for the pathophysiology directly relating the CNS lesion with the enzyme defect. This project was started as a single-laboratory size program to obtain a murine model for the human disorders related to phenylketonuria (PKU). Suspect heterozygote mutant offspring were generated using double-dose X-irradiation of mouse sires (see previous reports, M-NC-8, 1972-74). The offspring were screened for the defect in a manner similar to the human screening program, using blood plasma levels of phenylalanine (P), tyrosine (T), and their ratio (P/T) to distinguish abnormal, high values in possible mutants from a distribution of values obtained from mice with normal phenylalanine hydroxylation (PH) systems here shown:



The scheme illustrates the complex nature of the enzyme systems governing the steady-state levels of the amino acids in the blood (two enzymes, a cofactor and a coenzyme). Thus, the screening program, in principle, would detect a deficiency in any component shown or a change in amino acid levels due to other causes. At the onset of the project the dogma indicated that dihydropteridine reductase (DPR) was in such excess as to permit normal levels of hydroxylation in the heterozygote mutant deficient in this component. The program included, therefore, screening of a large sample of liver biopsies for direct measurements of DPR in the liver. Methods for screening for total dihydrobiopterin in hepatic samples were also used in this project.

The current thrusts of the project are to achieve a pharmacological model for the suspect PKU mouse in order to obtain the proper criteria for screening, and to utilize the difference in susceptibility to m-fluorophenylalanine (mFPA) (shown in rats) of normal and PH deficient animals as a possible screening method for PKU heterozygotes. A method for selection of phenylalanine hydroxylation-deficient mice by using a drug whose metabolism utilizes the PAH system to form a highly toxic mitochondrial poison, fluoroacetate, is being studied. It had been shown by others that rats treated with parachlorophenylalanine (pCPA) were protected from the lethal effects of high doses of the convulsant drug for 8-11 hours. pCPA is long-known to induce a state of about 95% PH deficiency in the rat for 24-72 hours

after a single injection of about 360 mg/kg. Attempts to induce a similar state in mice had failed to produce more than 50% inhibition of PAH for short periods, at the same or double that dose. This project applied various modes of administration of pCPA to mice, and measured the time course of uptake of the agent into blood plasma and liver, with corresponding in vitro enzyme assays for hepatic PAH. A program for separation of phenylalanine, tyrosine and pCPA from blood plasma and liver extracts at various times after administration of reagent was developed for the automated amino acid analyzer in collaboration with Gladys Deibler, LCM, NIMH. Protein contents of liver samples were determined by colorimetric analysis. Dose response curves for mFPA in the C57BL/6N mouse were obtained by intraperitoneal injection of mice. Comparison of lethality at several levels of PH was made at a mFPA lethality level of about 80%. Various other pretreatments which were expected to interfere with PH were used in attempts to achieve a less than 50% in vitro measurable hepatic PAH level.

Results showed that the difference between the rat and mouse in response to pCPA was due to a difference in uptake and disposal of the agent. Eighteen hours after the administration of 360 mg/kg pCPA in mice scant amount is detected in the blood or liver. The maximum level is reached at 3-6 hours and falls. Doubling the dose leads to approximately twice the maximal concentration in liver and blood from 3-6 hours and falling close to zero by 18 hours. The animals excrete large quantities of phenyl ketones during the 12 hour period. In vitro measurable PAH levels fall during the 6-18 hour period, using the natural cofactor (BH_4) or the synthetic ($DMPH_4$), confirming our previous findings at 24 hours with $DMPH_4$ (see previous report N-MC-8 1974). The inhibition curve was bimodal between zero and 24 hours. The maximal decrease in hepatic PAH activity compared with normal during 0-36 hours was found to be 45-50%. Plasma tyrosine level decreased, phenylalanine increased and the P/T ratio increased 1.5-3 fold during the zero to 9 hour period.

At the 24 hour level of inhibition of PAH and PH, the dose-response for lethality due to mFPA was not shifted; the 50% deficient animals were, therefore, not less susceptible to the agent. A new mode of administration of pCPA was tried with the view towards achieving high levels of pCPA in the blood and liver over longer periods. This was successful. Maximal plasma levels were about 4 fold higher than previously (approaching the levels seen in rats, 1 mM) and persisted over 24 hours. One test of mPFA toxicity at 21 hours (animals were about 70% deficient in in vitro measurable PAH) showed a small shift in the dose-response, indicating some protection.

Quantitative study of the uptake of pCPA in mice using our mode of liquid-membrane (L-M) administration demonstrated that the P/T ratio rose rapidly to about 3.0 from 1.29 $\pm$0.22 for the vehicle controls, peaking at 6 hours and elevated up to 2 days. Since hepatic pCPA was high during the 9-24 hour period early challenge with mFPA was undertaken. Results of injection of mFPA at 3 and 5 hours after L-M administration of pCPA was contrary to expectation since no protection was evident in that the percent survival of an acute phase-time course was the same for control

and treated animals. It appears that the acute type of inhibition of PAH, that of direct interaction with the PH system seen in vivo, is not sufficient to alter the metabolism of the toxic phenylalanine analog over a sufficient time for effective protection. Furthermore, after the acute intoxication, another form of impairment of PH occurs which, at 24 hours, does affect the rate of metabolism of mFPA.

Recent data using C57BL/6J mice (because of the frequent illnesses and unreliability of the NIH-supplied C57BL/6N mice) indicates that there is a very narrow time range in which an appropriate dose of mFPA would lead to the death of all normals by a certain time after this agent, leaving the "protected" animals alive.

Continuation of the study of the remaining, aged nonfertile 4th generation of suspect DPR-mutant families exhibited diminished in vitro measurable PAH activity (25-80% of controls)(see previous reports MNC 8, 1972-76). It was further shown that in vitro measurable PAH activity was similar in young adults and aged exbreeders from the normal NIH colony. The data further confirm the original premise that small increases in P/T in mice is a reflection of diminished PH activity in vivo due to either DPR insufficienc or decrease in the in vitro measurable PAH. The suspect mutants, therefore, have been established to exhibit enzymatic and biochemical characteristics of the human genetic disorder of Dihydropteridine Reductase Deficiency. We explain the failure to maintain the substrain by the normal reproductive methods as due to impairment of the hormonal regulations of mating, nesting, fertility and/or lactation/care behavior in mice since both male and female affected animals often survived with the deficiency beyond one year of age.

The results are significant to an understanding of the effects of pCPA in mice and modes of inhibiting PH in vivo in mice. The methods and results will be reported in a joint manuscript with Gladys Diebler, LCM, NIMH. Parameters of PH insufficiency in mice such as P/T ratio are now quantitatively related to the degree of impairment of hepatic PAH. Results confirmed the value we have used of 1.6 and over as suggestive of heterozygosity for "PKU" in suspect mutant mice. P/T values of about 2.0 are very elevated in this species where values greater than 3.0 have never been observed in PH deficient animals.

Attempts to determine a dose of mFPA with a corresponding time of administration after pCPA to screen for the unique survival of the "heterozygotic PKU mouse" have failed thus far. Results indicate that a dose high enough to produce death within 12-18 hours (risking the death of a potential heterozygotic PKU mouse) might better distinguish the possible mutant. Treatment with phenobarbitol to alleviate the convulsions could then lead to survival of the "pharmacological PKU individual". An all or none selection based on mFPA toxicity would enable the screening of large numbers of animals at 8 weeks of age. However, the principle appears to be impractical in mice at this time without further study.

The studies of murine DPR deficiency will be continued, time and personnel permitting. Preliminary results obtained last year provides a potent inhibitor for DPR. This drug could be used to produce a pharmacologically-induced state of DPR insufficiency in mice; the change in hepatic PAH level and/or CNS effects could be studied.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do **NOT** use this space)

U.S. DEPARTMENT OF
HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 01081-08 LNP

PERIOD COVERED

October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Cerebral Control of Movement

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	E. V. Evarts	Chief, Lab. of Neurophysiology	LNP NIMH
	Von A. Jennings	Guest Worker	LNP NIMH

COOPERATING UNITS (if any)

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INSTITUTE AND LOCATION

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.63	.92	2.71

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project involves utilization of single neuron recording techniques in behaving monkeys to study brain mechanisms underlying volitional motor performance. It has been shown that the laws of reflex action known to operate at the level of the spinal cord also operate at the level of the cerebral cortex. Motor cortex pyramidal tract neurons (PTNs) are impinged upon by afferent inputs which constitute the incoming limb of a transcortical loop. Thus, the phylogenetically new pyramidal tract system of the mammal is subject to the same laws of reflex action that characterize the phylogenetically older components of motor control systems. In addition to being driven by a servo system which stabilized movement and posture, PTNs are driven by a second major set of inputs, and it is this second set of inputs that underlies internally generated motor programs. These programs, reaching the motor cortex from the thalamus, are themselves a product of activity in basal ganglia and cerebellum.

Project Description:

The studies of movement in sub-human primates utilize two major procedures.

1. Electrical recording. Microelectrodes are used to record the activity of individual neurons during natural, voluntary movement in the monkey. This use of single unit analyses in relation to voluntary movement is analogous to the long-established use of single unit analysis in determining how the brain receives and elaborates its sensory input.

2. Behavioral training. The current work on this project is concerned with the properties of motor cortex neuron discharge in relation to the interaction of central programs and peripheral feedback. For these experiments, monkeys have been trained to make arm movements in order to maintain alignment between two lamps. The paradigm is one of "pursuit tracking." There are two visual displays--one lamp being the target (under the experimenter's control) and the other lamp (under the monkey's manual control) providing a feedback signal to the monkey. In this paradigm, the monkey viewed two horizontal rows of lamps (track and target lamps), each lamp in the track row being directly above a corresponding lamp in the target row. At all times only one lamp in each row was on. Output from a position transducer coupled to the handle selected which of the track lamps was illuminated. While the track display informed the monkey of the actual handle orientation, the location of the target lamp was programmed by the experimenter and indicated how the handle should be positioned.

Our work has used the experimental paradigm described above in demonstrating the relationship between centrally programmed volitional movement and feedback reaching motor cortex via the receptors of the moving part. In this work there has been special attention to motor cortex responses elicited by kinesthetic inputs during precise fine movement. These responses during precisely controlled movement were compared with responses to kinesthetic inputs during postural stability (when the monkey was seeking to maintain the handle steadily in one position) and during ballistic movement (when the monkey was seeking to make a rapid large displacement). In these studies over 700 neurons were analyzed. In about 30% of these neurons significant changes of activity were detected for both small and large movements, while another 30% changed exclusively in relation to the large fast ballistic movements. All units discharging with small movements also discharged with larger movements, but with the larger movements many additional PTNs which had been inactive with the small movements were recruited. Those PTNs which became active even with the smallest movements were also the most sensitive to peripheral inputs arising from limb receptors. Almost invariably, there was intense reflex activation of those units (especially the tonically discharging PTNs) most involved in control of precise small movements. A particularly striking result obtained in this experiment was a heightened sensitivity to kinesthetic inputs during precise fine movement. It has thus been shown that a highly sensitive transcortical loop operates during precisely controlled movement. But does this loop have properties consistent with servo control? To answer this question, let us begin by considering some of the a priori expectations

which one might have for PTN discharge in relation to movement, assuming that the PTNs are summing points in a transcortical servo loop. For PTNs which are summing points in such a loop, realization of the action resulting from discharge of the PTNs should tend to reduce (i.e., to inhibit) discharge, whereas failure of realization should tend to increase (i.e., to excite) discharge. A familiar circuit exhibiting such negative feedback is the loop involving muscle receptors and alpha-motoneurons (α-MNs). Within this loop, the consequences of α-MN discharge (decreased muscle length and/or increased muscle tension) feedback onto the α-MN so as to reduce α-MN discharge:

↓ muscle length → ↓ muscle spindle discharge → ↓ α-MN discharge
↑ muscle tension → ↑ Golgi tendon organ discharge → ↓ α-MN discharge

Motor cortex PTNs appear to be in a loop analogous to the one impinging on spinal cord α-MNs, a loop carrying information such that externally induced (i.e., passive) movement corresponding to the active movement called for by the PTN inhibits the PTN's discharge. By analogy with the model of negative feedback presented for the α-MN, an external "assist" which helps to realize the movement "commanded" by the discharge of the PTN will inhibit its discharge, whereas application of a force which opposes this movement will excite the PTN.

Central Programs Can Override the Servo

The servo-controlled neuronal discharge described above underlies operation of an automatic system which is of critical importance in stabilizing our finest most precise movements. But what happens when a subject wishes to carry out a movement requiring muscular activity opposite to that which would be called for by the servo underlying stability? What happens when we wish to make a movement which contradicts the reflex inputs which are "hard-wired" to elements of our motor control systems? How does a subject execute an instruction calling for a motor act which runs counter to reflex drives? In order to get information on this point, we have studied PTN responses in monkeys trained to react to a limb displacement according to a prior instruction and regardless of the direction in which the perturbation displaced the limb. A key goal in the design of this experiment was dissociation of the reflex from the intended components of motor activity. It was in this respect that this study in monkeys differed from previous experiments in monkey or in man. To achieve this dissociation, either of the two possible instructions (ins-pull and ins-push) was followed by either of the two possible directions of perturbation (per-pull and per-push). For example, when a monkey was given an instruction to push (ins-push) in response to a subsequent perturbation of its arm, the perturbing trigger could move the arm in either of two possible directions: either toward the monkey (per-pull) or away from the monkey (per-push). When ins-push was followed by per-pull (a perturbation which stretched triceps and opposed the intended push movement), the segmental reflex response in the stretched triceps muscle was of the same sign (+) as the intended response (+), and there was association of the reflex and intended components of the muscle response. But when ins-push was followed by per-push, a triggering

perturbation which shortened triceps and assisted the intended push movement, the segmental reflex response (-) in the shortened triceps muscle was opposite to the intended response (+): now the segmental reflex response of triceps (to becoming shorter while its antagonist was being stretched) was to become less active, and the triceps discharge which occurred when the monkey carried out the intended push movement was in spite of rather than because of segmental inputs.

In summary, it was seen that arm area PTN discharge associated with a contralateral arm movement triggered by an input to the responding arm has two components: 1) a relatively short-latency reflex component which depends on the nature of the input to the arm, and 2) a longer latency component which depends on the movement that the subject intends to perform. The first component of discharge forms part of a transcortical servo-loop, while the second component of PTN discharge is related to the nature of the intended output and would seem to depend on a central program rather than on the nature of the input which triggers this program. When an excitatory perturbation triggers a movement involving quiescence of the PTN, only the first (or reflex) component of discharge occurs. Conversely, when a movement involving discharge of the PTN is triggered by a perturbation which suppresses activity of the PTN, only the second (or intended) component of PTN discharge occurs.

Proposed Course of Project:

Having demonstrated the existence of a transcortical servo loop which is of special importance in precise finely controlled movement, we will seek to determine the sources within cerebral cortex from which the sensory driving of the motor cortex servo arises. Recent anatomical studies by other investigators have shown that strong fiber connections pass into motor cortex from sensory and associative regions in the post-central gyrus. Our work in the coming year will seek to examine the neuronal activity in these post-central areas and determine the extent of which the servo driven activity in motor cortex might arise within these regions. It is our hope that it will be possible to develop techniques for reversible cooling of the areas in question to ascertain the extent to which disruption of the transcortical servo will disrupt precisely controlled motor performance. As part of this study, it would be our intention to record activity of motor cortex neurons driven by the servo during precise motor control and to determine the extent to which such servo driving would be blocked by reversable cooling of the proposed relay station in the post-central areas 2 and 5.

Significance to Biomedical Research and to the Program of the Institute:

This project seeks to apply basic research results on central control of movement to an understanding of normal and abnormal movements in man. To the extent that the project succeeds, it will contribute to the research goals of the NIMH.

We have shown that the laws of reflex action, long known to operate at the level of the spinal cord motoneuron, also operate at the level of the cerebral

cortex. Motor cortex PTNs are impinged upon by afferent inputs which constitute the incoming limb of a transcortical servo loop. Thus, the phylogenetically new pyramidal tract system of the mammal is subject to the same laws of reflex action that characterize phylogenetically older components of motor control systems. But in addition to being driven by a servo system which stabilizes movement and posture, PTNs are driven by a second major set of inputs, and it is this second set of inputs that underlies internally generated motor programs. These programs, reaching the motor cortex from the thalamus, are themselves a product of activity in basal ganglia and cerebellum. The results of these experiments are of value in helping us to formulate mechanisms involved in the disorders of movement associated with diseases of the basal ganglia. It is in patients with such disorders that one clearly sees the contrast between voluntary and involuntary movement. The patterns of muscular activity occurring in association with the involuntary movements of certain basal ganglia disorders may be similar to the patterns associated with normal voluntary movement. The difference between the voluntary movements of the healthy individual and the involuntary movements of patients with Gilles de la Tourette's syndrome on Huntington's disease are two-fold: 1) the involuntary movements in the neurological disorders are purposeless, and 2) the patients exhibiting these involuntary movements are unable to prevent them.

Of the two major classes of inputs impinging upon the cerebral motor cortex and generating the stream of impulses passing via the pyramidal tract of the spinal cord, that class of inputs which operates automatically and constitutes the afferent limb of the transcortical servo seems the simplest to understand. This transcortical servo operates according to Sherringtonian principles of reflex action. The second set of inputs passing via the thalamus to the motor cortex depends upon basal ganglia and cerebellum, and is much more complicated. Thus, in order to understand voluntary movement we need to comprehend the sorts of information processed by cerebellum and basal ganglia and to discover the way in which the outputs of cerebellum and basal ganglia interact in the thalamus.

Publications:

Fromm, C. and Evarts, E.V.: Relation of motor cortex neurons to precisely controlled and ballistic movements. Neurosci. Ltrs. 5: 259-265, 1977.

Evarts, E.V. and Fromm, C.: Sensory responses in motor cortex neurons during precise motor control. Neurosci. Ltrs. 5: 267-272, 1977.

Evarts, E.V.: Mediation of quick motor responses by motor cortex pyramidal tract neurons in the monkey. Neuroscience 3: 95-98, 1978.

Tanji, J. and Evarts, E.V.: Anticipatory activity of motor cortex neurons in relation to the direction of an intended movement. In: Functional Significance of the Brain Electrical Processes. (published in Russian). Moscow, Nauka Press, 1977, pp. 218-225.

Evarts, E.V. and Fromm, C.: The pyramidal tract neuron as summing point in a closed-loop control system in the monkey. In Desmedt, J.E. (Ed.): Cerebral Motor Control in Man: Long Loop Mechanisms, Progress in Clinical Neurophysiology, vol. 4. Basel, Karger, 1978, pp. 56-69.

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PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 MH 01082-13 LNP

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October 1, 1977 to February 28, 1978

TITLE OF PROJECT (80 characters or less)

Muscle Function and Metabolism

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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.46	.21	.25

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

We have shown that fatigue, in response to tetanic stimulation in single muscle fibers of the frog, is not due to depletion of ATP, the energy source for contraction, but is due to interference with excitation-contraction coupling and the ability of the muscle to utilize available ATP.

PHS-6040
(Rev. 10-76)

Project Description:

Single muscle fibers of the frog semitendinosus were stimulated repetitively, and tension and metabolic profiles were measured and related to each other.

Results:

The experimental work on this project was completed in the prior year, and the results were collated and prepared for publication this year. The results indicate that fatigue in single fibers, in response to tetanic stimulation, is not due to depletion of ATP, the energy source for contraction, but is due to interference with excitation-contraction coupling.

Proposed Course:

The project was terminated in February 1978 when the principle investigator (S. Rapoport) moved to the National Institute of Aging.

Publications:

None.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER ZO1 MH 01083-12 LNP

PERIOD COVERED

October 1, 1977 to February 28, 1978

TITLE OF PROJECT (80 characters or less)

Transport Mechanisms Across Membranes and the Blood-Brain Barrier

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	S. I. Rapoport	Neurophysiologist	LNP NIMH
OTHER:	K. Ohno	Visiting Fellow	LNP NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Neurophysiology

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.92	1.04	.88

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Radiotracers of various nonelectrolytes were injected intravenously in conscious rats, and arterial plasma concentrations were determined as a function of time until the animals were killed by decapitation. Regional brain concentrations of tracer were measured. Nonelectrolyte permeability at the blood-brain barrier is due to simple diffusion through the cerebral vasculature, and is comparable to permeable across nonporous, bimolecular lipid membranes.

Results:

The data were fit by computer to a multicompartmental kinetic model which allowed calculation of cerebrovascular permeability of tracers as a function of their physical-chemical properties (charge, molecular weight, lipid solubility). Cerebrovascular permeability of nonelectrolytes was shown to be a function of lipid solubility and molecular weight, indicating that non-electrolytes passed through the blood-brain barrier by a simple diffusion mechanism, just as they cross bimolecular lipid membranes. The results are being prepared for publication.

Proposed Course:

This project was terminated in February 1978, when the principal investigator (S.I. Rapoport) moved to the National Institute of Aging.

Publications:

None.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 01084-07 LNP

PERIOD COVERED

October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Eye-Head Coordination in the Primate: Neural Substrate and Plasticity

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	F. A. Miles	Visiting Scientist	LNP NIMH
OTHER:	D. J. Braitman	Guest Worker	LNP NIMH
	S. J. Lisberger	Staff Fellow	LNP NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Neurophysiology

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
5.53	2.58	2.95

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The long-range purpose of this project is to examine the neural basis of the adaptive mechanisms responsible for maintaining appropriate performance levels in the vestibulo-ocular reflex. Our present concern is to determine where the modifiable elements are located through single unit recordings in the vestibulo-ocular reflex pathways of (a) normal monkeys and (b) monkeys whose vestibulo-ocular reflex has been modified as a result of prolonged optical reversal of vision. No significant differences were found between the two groups of animals in the gain, phase or resting discharge rates of their semicircular canal primary afferents, hence, vestibular efferents are not involved in the adaptive process. Signals carried by the Purkinje output cells in the floccular lobes of the vestibular cerebellum were different in the two groups of monkeys but the nature of these changes suggested that they were incidental to modifications occurring elsewhere in the vestibulo-ocular pathways.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives:

Chronic single unit recordings were carried out with the object of locating the site of the neural modifications which underlie the adaptive capability of the vestibulo-ocular reflex (VOR). Our initial concern is to find the point in the vestibulo-ocular pathway at which changes are first manifest.

Methods Employed:

Single unit recordings were made in the vestibular nerve, flocculus, and vestibular nuclei of (a) normal monkeys and (b) monkeys whose VOR had been modified by prolonged exposure to various optical devices (dove prisms, telescopic spectacles and fixed-field spectacles). Monkeys were seated in a special chair which could be oscillated about the vertical axis by a servo-controlled torque motor. Since the animals' heads were secured to the chair, the chair rotation provided controlled vestibular stimulation. The vestibular sensitivity and phase of individual neuronal responses was calculated from the discharge modulations accompanying sinusoidal oscillations of the chair at varying frequencies. Our previous experiments have shown that the discharge of semicircular canal primary afferents is not immediately influenced by ongoing visuo-vestibular experience, but the concern of the present experiments was to look for long-term influences. For the recordings in the flocculus, it is necessary to use monkeys trained on a fixation task, since this area carries oculomotor as well as vestibular signals. Thus, output cells from this region carry both head velocity (vestibular) and eye velocity signals. Special paradigms were devised to dissociate these normally co-varying signals and hence permit quantification of each separately. There are many different cell types in the vestibular nuclei, but the present recordings were concerned only with those carrying vestibular signals (possibly together with some phasic activity associated with rapid eye movements). All data were recorded on analog magnetic tape and subsequently digitized for quantitative analysis on a PDP-12 computer.

Major Findings:

Quantitative analyses of single unit recordings from the vestibular nerve and flocculus strongly suggest that the modifications underlying the adaptive capability of the VOR do not occur in either the labyrinth or the flocculus.

1. The rotational sensitivity of some 437 fibers in the vestibular nerve innervating horizontal and vertical semicircular canals were quantitatively examined. Approximately half of these fibers were recorded in normal monkeys and the remainder in monkeys whose VOR gain was either very low (less than .2) or actually reversed. Statistical analyses revealed no significant differences between normal and low gain animals in the gain, phase, or resting discharge rates of their semicircular canal fibers. We conclude that the gain changes are not mediated by the vestibular efferent system.

2. A total of 532 Purkinje output cells were characterized in the flocculus of normal and low gain monkeys in terms of their vestibular and oculomotor-related discharges. Approximately three-quarters of these cells carry eye velocity and head velocity signals (called Gaze Velocity Cells), of which approximately one-third relate to vertical eye and head movements and two-thirds to horizontal. The horizontal Gaze Velocity Cells were quantitatively examined for VOR gain related changes. In the normal monkey, the eye and head velocity signals carried by these cells have the same directional polarity (ipsilateral), approximately the same strength (head signal ÷ eye signal = 0.99) and sum together approximately linearly. In the low gain animals there is a change in the relative strengths of these head and eye velocity signals (head signal ÷ eye signal = 0.68). If the modifiable synapses responsible for changing the gain of the VOR were located in the flocculus, then one would have predicted that the ratio head signal/eye signal would increase in the low gain animal. The fact that it decreased suggests to us that (a) the modifications responsible for reducing the VOR are not located in the flocculus and (b) the flocculus receives a modified input. This interpretation would mean that the changes observed in the flocculus are a secondary consequence of modifications occurring elsewhere in the vestibulo-ocular pathway.

3. Recordings have been initiated in the vestibular nuclei, and approximately 250 cells with a vestibular signal have been recorded from one animal in the low and high gain state; however, more data are required before any definitive statement can be made about possible changes there.

Significance to Biomedical Research and the Program of the Institute:

The single unit recordings are being undertaken to establish the exact locus of the modifiable elements in the VOR. A great deal is known about the anatomy and physiology of the VOR, which represents one of the best examples of adaptive plasticity currently available in the primate brain. Once we know where the modifiable elements are located, a whole new area of research related to aspects of motor learning will be opened up.

Proposed Course:

Studies will continue to search for the locus of the modifiable elements in the VOR, pursuing the problem stage by stage through the vestibulo-ocular pathway. Since appropriate changes were not seen in either the vestibular nerve or the flocculus, we shall now concentrate our efforts on the vestibular nuclei.

Publications:

None.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 01089-02 LNP

PERIOD COVERED

TITLE OF PROJECT (80 characters or less)

Cortical Response to Sensory Input as a Function of Steady State Motor Activity

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. R. Wolpaw	Staff Fellow	LNP NIMH
Other:	T. R. Colburn	Chief, Section on Technical Development	IRP NIMH

COOPERATING UNITS (if any)

Section on Technical Development, IRP, NIMH

LAB/BRANCH

Laboratory of Neurophysiology

SECTION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

INSTITUTE AND LOCATION

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	0.75	1.25

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The project is a study of the responses of single units in primary motor cortex (area 4), primary sensory cortex (areas 1, 2, and 3), and areas 5 and 7 of macaca mulatta to wrist flexor and extensor torque pulses or isolated muscle stretch superimposed on a range of steady torques. The aim is to determine the relationships between cortical response to torque pulses or isolated muscle stretch and steady state torque and the relationships between cortical response to torque pulses or isolated muscle stretch and unit behavior during steady state torque. In connection with this project we have developed a technique which permits stretch of individual muscles in the awake behaving primate by direct application of calibrated force unaccompanied by other stimuli. An external electromagnet applies force to a small piece of permeable metal chronically imbedded in the musculotendinous junction. We are using it to provide relatively pure muscle receptor stimulation, in contrast to torque pulses which stimulate muscle, joint, and cutaneous receptors.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives:

The sensory-motor cortex of man and subhuman primates may be conceived of as part of a servo-system, generating outputs leading to movement and in turn receiving sensory feedback resulting from movement. The feedback has a major role in determining subsequent cortical output. Thus understanding of how cortical neurons participate in motor control requires understanding of how cortical response to sensory feedback is related to the movement underway. The objective of this project is to test the hypothesis that the signs and strengths of the feedback signals returning to neurons in sensory-motor cortex are systematically related to the signs and strengths of the output signals generated by these neurons in the course of movement performance. In the experiment transient feedback signals are presented to a monkey while the steady state motor activity required of the monkey is varied. Delineation of the relationships between neuronal responses to these transient feedback signals and the concurrent steady state motor output is the goal of the study.

Methods:

The monkey is rewarded for maintaining a straight wrist posture, neither flexed nor extended. A torque motor generates forces which oppose the efforts of the monkey. The direction and magnitude of the force generated by the torque motor thus dictates the direction and magnitude of the opposing force which must be generated by the monkey. The monkey is rewarded for exerting a force equal and opposite to the force generated by the motor. The force generated by the torque motor may be varied over a large number of levels ranging from a level requiring strong wrist extension by the monkey to one requiring strong wrist flexion by the monkey. Two types of transient feedback stimuli may be delivered: flexor or extensor 50 msec torque pulses generated by the torque motor, and 100 msec isolated muscle stretches. To allow the isolated muscle stretch, a technique has been developed involving implantation of a small coated iron slug into the musculotendinous junction of flexor carpi ulnaris and application of force to this slug and thus to the muscle via an external electromagnet.

Following training of the monkey single unit activity is studied in cortical areas 4,1,2,3,5 and 7. Unit activity, handle position, and stimulus codes are recorded and analyzed off-line by a PDP-12 computer. For each unit, the analysis focuses on the frequency and pattern of discharge at each steady state force level, the change in frequency and pattern of discharge produced by torque pulses or muscle stretch at each steady state force level, and the relationships between torque pulse or muscle stretch responses and behavior at the steady state force levels.

The brain is marked grossly at the time of death; gross and microscopic examination is performed to determine the state of the cortex and to correlate penetrations with areas of cortex.

Findings:

Data have been obtained from over 1800 units in four monkeys. The primary interest is in those units which respond within 60 msec, that is, units which are clearly responding to the torque pulse prior to change in the direction of handle movement due to rebound. Full analysis of the results is not as yet completed. Analysis of responses of those units tested with both torque pulses and isolated muscle stretch indicates that over 60% of the units in areas 1 and 3, and 45% of those in area 4 which respond to torque pulses also respond to isolated muscle stretch, and that the responses are very similar in form and latency. In these units, the intensity of the response to isolated muscle stretch is a considerable fraction of the intensity of the response to torque pulses. These results indicate that muscle receptor input has a major role in the response to pertubations occurring during skilled activity.

Preliminary analysis of the units in areas 5 and 7 indicates that those units whose response to a torque pulse of given polarity varies with the steady state torque usually give a greater response when the steady state torque is of the same polarity. These data appear to suggest a positive correlation between the magnitude and polarity of steady state motor output and the magnitude and polarity of cortical unit response to sensory input. Further analysis of the data from these areas as well as areas 4,1,2, and 3 is proceeding.

Development of the technique for electromagnetic muscle stretch continued with exploration and presentation of the relevant electromagnetic theory, improvement in slug and design, and alteration of the coil to allow greater force with less current.

Significance:

Further elucidation of how relevant sensory feedback is utilized in ongoing performance of skilled motor activity is essential to better understanding of motor control. Some understanding of this problem has been achieved on the spinal cord level in the relationships described between alpha motoneuron activity and gamma motoneuton activity and muscle spindle responsiveness. This project is an attempt to begin delineation of analogous relationships on the cortical level, to determine how the cortical response to relevant sensory input during motor behavior is related to that behavior.

Proposed Course:

Full analysis of the data is in process and will be completed in three to four weeks, thus allowing delineation of conclusions indicated by the data in regard to the relationships between cortical response to sensory feedback and ongoing motor output. When these results are written up, the project will be terminated.

Publications:

Wolpaw, J.R. and Colburn, T.R.: Electromagnetic stretch of individual muscles in behaving primates. Brain Research 141: 193-196, 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 01090-02 LNP

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)
CNS Connections Studied by Amino Acid Autoradiography and Horseradish Peroxidase

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Miles Herkenham Staff Fellow LNP NIMH

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Neurophysiology

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.4	1.0	1.4

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A neuroanatomy research laboratory was made operative this year and has been used for studies of a) neuronal connections in the brain and b) localization of brain sites which are selectively involved in sleep and drug-induced sedation. The newly developed fiber tracing techniques based on tritiated amino acid autoradiography and horseradish peroxidase (HRP) histochemistry have been used to demonstrate neural pathways arising from intralaminar and midline thalamus projecting to widespread areas of cerebral cortex and terminating in distinct laminar patterns. These data provide structural evidence for mechanisms of thalamocortical integration that have not previously been appreciated. In another form of autoradiography, labeled biologically active substances, tryptophan and diazepam, are systemically injected so that their final brain distribution can be mapped. The localization of the active uptake sites may lead to a better appreciation of the functional properties of tryptophan as a precursor to serotonin synthesis (which may be important in sleep mechanisms) and of diazepam as a pharmacologically potent tranquilizer, anticonvulsant and muscle relaxant.

PHS-6040
(Rev. 10-76)

Studies of CNS Connections

Objectives:

This project uses histochemical tracing techniques to map neuronal connections within the brain. Work in progress has dealt with the cortical projections of thalamic nuclei, which collectively may be called the "nonspecific" thalamus. The cell groups under investigation--the midline, intralaminar, parafascicular, ventromedial and ventrolateral nuclei--attracted interest over 30 years ago when electrophysiologists found that electrical stimulation of nonspecific thalamic sites in the cat could elicit widespread and synchronous cortical responses. The data further suggested that the pathways carrying these electrical events to the cortex were not only widely distributed, but were anatomically separate from the known thalamocortical pathways originating in the "specific" thalamic relay nuclei. Moreover, microelectrode depth recordings suggested that specific and nonspecific afferent fibers terminate in separate cortical layers. Unfortunately, however, the neuroanatomical tract tracing techniques, then based on degenerating axons, could find little evidence for nonspecific thalamocortical projections terminating in wide areas and in unique laminar patterns. Consequently, the early workers abandoned their interest in the possibility that these mechanisms could in part underlie cortical and behavioral arousal phenomena, and even conscious integration. The present studies have provided the long-sought anatomical bases for the electrophysiological phenomena.

Methods Employed:

The techniques of anterograde fiber tracing by tritiated amino acid autoradiography and location of cells of origin by retrograde axoplasmic transport of HRP, both developed in the last decade, have provided more sensitive and artifact-free data than the older degeneration methods. Moreover, the deposition of the tracer enzymes in the brain by microiontophoresis produces injection foci that are much smaller, yet more concentrated, than those produced by hydraulic pressure. The combination of these newly developed methods has made possible the aforementioned research.

Most of the work done this year has been autoradiographic. Essential facilities were installed in August 1977 and have been in full operation since then. In addition, a technician has been trained to perform careful and ultra-clean histology necessary for producing good autoradiographic material.

The anterograde data can be corroborated by using the HRP technique to locate cells of origin of widespread thalamocortical projections. Placement of the retrograde marker into various cortical regions will label nonspecific thalamic neurons only if the histological procedure is maximally sensitive. Efforts are in progress to insure that reliable, sensitive results can be obtained. Several staining protocols can be followed to bring out the visible HRP reaction products, and some call for more sensitive and safer (i.e., noncarcinogenic) substrates than others. Since these are the more recently developed methods, certain reaction vicissitudes must be eliminated by

experimentation with the procedure. Consideration has been given to the likelihood that the technique will later be used for studies of the monkey brain, which presents additional histological problems because of its larger size.

Major Findings:

Previous work by the principal investigator utilizing the autoradiographic technique has shown a remarkable and unique projection to the most superficial layer of almost the entire neocortex originating from the ventromedial thalamic nucleus in the rat. Another widespread and hitherto unrecognized projection to the hippocampus and limbic cortices was found after placing a mixture of ^{3}H-leucine and ^{3}H-proline into the nucleus reuniens of the thalamic midline group. Results from autoradiographic cases of isotope injection into intralaminar thalamic nuclei suggest another instance of widespread neocortical distribution but, in contrast to that of the ventromedial nucleus, intralaminar projections terminate in deep cortical layers. Some results suggest that thalamic inputs may be distributed to two or even three layers, and current experiments are aimed at determining whether the multiple and spaced laminations arise from a single or adjacent thalamic cell groups. Further analyses will also provide the topography of the thalamocortical and thalamostriatal projections. Must of this information can be acquired from HRP case material.

An unexpected finding from the control cases, in which the ventroanterior and ventrolateral nuclei were injected, is that those nuclei previously thought to project to the motor cortex appear to have a much wider cortical distribution. Autoradiographic and HRP experiments are now underway to map the projections of this additional thalamic complex, which is also associated with the nonspecific projection system. Interest in the inputs to motor cortex may generate research in these same systems in the monkey, thereby providing a bridge between the anatomical work in the rat and some ongoing and planned electrophysiological/anatomical studies concerning the primate motor cortex.

Studies of CNS Functional Neuroanatomy

Objectives and Methods:

The uptake of tracer amounts of ^{14}C-labeled 2-deoxy-D-glucose (2DG) by brain tissue reflects glucose utilization of a particular region (see Project No. ZO1 MH 01085-04 LNP for the method). A general lowering of brain metabolism by anesthetics occurs for most brain areas, but in this project it has been found that an anatomical system consisting of the habenula, interpenduncular nucleus and the habenulo-interpenduncular tract, shows relatively much greater labeling by ^{14}C-2DG during anesthesia. This demonstrates altered functional activity in an anatomically defined neuronal network including the interconnecting myelinated fiber tract.

A related methodological approach may be used to demonstrate other changes of local regional uptake of functionally significant labeled compounds. Two such projects have been initiated. The purpose of the first is to further examine

the role of the suprachiasmatic nucleus and several other brain regions in mechanisms of circadian rhythmicity. Experiments using the 2DG method (Project No. Z01 MH 01085-04 LNP) have demonstrated that the suprachiasmatic nucleus acts as a "biological clock" whose metabolic activity is regulated by light-dark cycles but which shows rhythmical activity even in constant darkness.

This nucleus has connections with other brain regions by which it may affect the timing of cyclic behaviors and endocrine systems, and is rich in serotonin, which is thought to play a role in sleep. Demonstration of alterations in the incorporation of radioactive tryptophan into serotonin-containing neurons according to the circadian rhythym may help us to better understand how the cyclic regulatory influences are generated. In the experiments just underway, therefore, ^{14}C tryptophan will be injected into rodents at various points in their circadian cycle. The subjects are hamsters, selected because they show rigid behavioral adherence to the light-dark cycle. If serotonin plays a role in sleep mechanisms, then differential amounts of radioactivity may be found in specific brain regions of animals injected with ^{14}C-tryptophan either in the dark period or light period. A positive result will not only support the notion of a significant role of serotonin synthesis in sleep mechanisms, it will show which brain regions are important for that role.

The purpose of the second project is to locate the brain regions that bind ^{3}H-diazepam. The benzodiazepines are a class of drugs of which diazepam, or Valium, is the best known. They are known to be pharmacologically active in humans and are important clinically as tranquilizers, anti-convulsants and muscle relaxants.

Quite recently, experimental work in vitro has shown that the brain contains sites that bind diazepam. Further work has shown that the brain may contain its own endogenous form of this substance. A better understanding of the mechanisms of diazepam action will require knowledge of the specific brain regions that contain the diazepam binding sites.

The experiments just underway are a collaborative effort with Dr. Phil Skolnick. ^{3}H-diazepam was injected systemically into rats. The animals were sacrificed after a period during which the drug became bound to the receptive brain sites. These brains will be processed for autoradiography in a manner quite similar to that used for the studies of CNS connections. If modifications are required, the procedure likely to be followed will be for autoradiography of diffusable substances, such as for 2DG.

Significance to Biomedical Research and to the Program of the Institute, and Proposed Course:

The study of thalamocortical projections, when complete, will show more precisely the nature of thalamic terminations in the cerebral cortex. The newly developed neuroanatomical histochemical tracing methods, wherever applied, tend to reveal CNS connections in more detail and entirety than was previously demonstrated or imagined. In this case, they show that a traditional

concept--that thalamocortical fibers terminate mainly in the intermediate layers--must be revised in light of the demonstration that the cell groups lying outside the main thalamic relay nuclei have unique forms of laminar afferentation. Just as the "wiring plan" of the cerebral cortex must include the lamination of specialized cortical efferent cells, now the various types of laminar inputs must be included for a better understanding of cortical function.

Likewise, the great spread of these nonspecific thalamic projections over many distinct cortical regions suggests an important form of neural integration. The emerging possibility that the widespread projections arise from thalamic nuclei which are themselves innervated by components of the extrapyramidal system (substantia nigra, cerebellum, corpus striatum) suggests some role of movement in states of awareness and arousal. The demonstration of similar neural connections in higher mammals would be useful.

The study of the brain localization of radioactively labeled substances such as 2-deoxy-D-glucose, tryptophan and diazepam, is an emerging hybrid of recently developed histochemical and biochemical techniques. In each instance the knowledge to be gained permits new insights into functional neuroanatomy. Previous efforts to elucidate structure-function relationships were limited by the paucity of useful techniques. Autoradiographic localization of biologically and pharmacologically active substances provides a more powerful tool for observing how the central nervous system operates.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 01091-01 LNP

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Motor Function in Patients with Neuropsychiatric Disorders

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	E. V. Evarts	Chief	LNP NIMH
OTHER:	H. Teravainen	Visiting Scientist	LNP NIMH
	D. B. Calne	Chief	ETB NINCDS

COOPERATING UNITS (if any)
Experimental Therapeutics Branch, NINCDS

LAB/BRANCH
Laboratory of Neurophysiology

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.20	2.0	1.65

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The relevance of movement disorders to neuropsychiatric illness has become increasingly apparent with the discovery that symptoms of Parkinson's disease may occur as side effects during therapeutic administration of neuroleptic drugs and that cessation of neuroleptics may be followed by tardive dyskinesia. The potential value of information concerning such neurochemically determined motor disorders is twofold: 1) at an applied level, quantitative measures of motor function will be useful in drug dosage regulation of therapeutic agents and in drug effectiveness comparisons for different agents; 2) at a basic level, discovery of mechanisms of motor disorders will help us to understand the brain disturbances underlying the wide range of neuropsychiatric illnesses related to neurochemical disturbances. Studies thus far have focused on obtaining quantitative indices of the fluctuations in effectiveness of anti-parkinsonian drug therapy. Tests of movement speed and reflex responses to limb displacement have provided us with measures which correlate with clinically determined fluctuations in drug efficacy, pointing to the feasibility of setting up routine tests of motor function to be administered in clinical settings.

Project Description:

Studies have been carried out in 80 NINCDS patients with Parkinson's disease (PD) and in normal subjects in three age groups: (1) 50-80, (2) 25-49 and (3) less than 25 years. Repeated observations have been obtained in individual patients to follow fluctuations of motor status occurring in the course of treatment with anti-parkinsonian drugs. The patients studied have in some cases been hospitalized because of "on-off" phenomena (i.e., the rapid changes from the dyskinetic to the rigid, bradykinetic state as drug levels change from "too much" to "too little"). Observations in these subjects have allowed valuable within-subject comparisons of quantitative measures of motor function.

It is widely agreed that the major cause of disability in PD is poverty of movement, and our studies have provided quantitative indices of speed of movement as a gauge of therapeutic effectiveness of anti-parkinsonian drugs. We have studied rapidly alternating movements carried out at a pace determined by the subject, and have also studied velocity of movement in a visual reaction time paradigm in which the subject maintains a stationary limb position until the appearance of a visual stimulus which calls for movement. Transducers measure movement velocity and EMG electrodes applied to the surface of the relevant muscles detect associated muscle activity.

Another feature of our procedure involves application of known displacements to the limb in order to elicit reflex responses and to relate changes in reflex responses to clinical status. In our procedures we have used an electronically controlled mechanical system for eliciting reflexes and obtaining quantitative indices of reflex responses. There is now evidence that basal ganglia disorders, while being associated with a normal short latency tendon jerk, are associated with alterations in longer latency responses to limb displacement and it is clear that in patients with basal ganglia disorders, small displacements of the limb may trigger sustained tremor. Understanding and quantitation of these responses to limb displacement will be useful in understanding the disease states in which these pathological phenomena occur.

Major Findings:

Studies of movement time and movement velocity in the visual reaction time paradigm have shown that movement time is a useful index of impaired function in PD. Patients with PD whose drug dosage is insufficient may have movement time elevations of the order of 500%. In contrast, normal subjects or PD patients whose drug level is adequate have movement times which are both low in value and tightly clustered with little variability. Thus, an elevated movement time is an index of malfunction and can be used to regulate drug dosage. The reliability of this measure points to the value of setting up routine procedures for regular measurements of motor function in PD patients, our goal being to make determinations as simple as taking the blood pressure or temperature.

Investigations of responses to limb displacement in PD patients and normal subjects have shown that PD patients with hypertonus exhibit enhanced responses to muscle stretch, but that enhancement of stretch reflexes also occurs in normal subjects with elevated resting EMG activity. Our normal subjects were subdivided according to age: 18-24, 25-49, and 50-80, and while subjects in the two younger age ranges exhibited very low levels of background EMG activity, several of the older normal subjects exhibited tonic EMG activity even when the steady state pre-stretch load (controlled by a torque motor) was such as to call for silence of the biceps muscle from which EMG recordings were obtained.

Responses to limb displacement were evoked by torque steps of 1 sec duration. Rigid PD patients exhibit (1) a higher level of pre-displacement EMG; (2) a smaller displacement in response to the change in torque, i.e., greater muscle stiffness; (3) a larger EMG reflex response to displacement. But findings similar to those shown by the PD patients are also seen in those older normal subjects with elevated muscle tone.

For quantitation of EMG responses to displacement, rectified EMG activity is integrated for a 250 msec period before and for 10 successive 25 msec periods following limb displacement. The average value of EMG activity during the 250 msec control period provided a measure of resting muscle tone. Integrated EMG activity occurring in the period 25-50 msec following displacement provided an index of the tendon jerk amplitude, and amplitudes of longer latency reflex responses were given by integrated EMG values at latencies of 50-75, 75-100, and 100-125 msec. It was found that PD patients with high resting levels of EMG activity have elevated reflex responses, but that there is no elevation of reflexes in PD patients without hypertonus. Such a positive relation between stretch reflexes and level of muscle activity has long been known to exist, and mechanisms (e.g., alpha-gamma linkage) underlying this relationship are well understood.

In our work the stretching stimulus was a torque step rather than a displacement step, and the actual displacement generated by the torque step varied depending on muscle stiffness, which in turn was elevated in rigid patients. Thus, the displacements (and stretches) delivered to the rigid PD patients tended to be less than those delivered to control subjects. Work to be undertaken will involve use of the torque motor in a configuration allowing delivery of controlled displacements rather than controlled torques. It would seem clear that such a mode of stimulation is preferable, and that use of controlled displacements with careful adjustments of pre-displacement load (to control steady-state muscle activity) should make it possible to find out if rigid PD patients exhibit reflex enhancement in excess of that which would be expected on the basis of the enhanced resting muscle discharge which is commonly associated with rigidity.

Significance to Biomedical Research and to the Program of the Institute:

The results which have now been considered show that tests measuring speed of movement reflect actions of anti-parkinsonian drugs, and we believe that

utilization of such tests on a routine basis could provide data relevant to clinical management. Though the instrumentation we have used in studying movement in PD is complicated and costly, it is clear that the routine tests which we contemplate can be carried out with simple, relatively inexpensive equipment: computers, torque motors, EMG recording apparatus and visual display systems for pursuit tracking are quite unnecessary for quantitative tests reflecting parkinsonian bradykinesia. But the sophisticated instruments we have developed do hold promise in basic studies aimed at understanding the nature of the motor disorders associated with basal ganglia dysfunction (e.g., Huntington's disease, tardive dyskinesia, PD), and acquisition of this understanding should enhance our ability to diagnose and treat the disorders.

Proposed Course:

Work to extend these studies to patients with psychomotor disorders will continue. For this study the principal diagnostic groups will involve neuropsychiatric diseases in which dementia is a central symptom, such as Huntington's disease, Korsakoff's psychosis, Alzheimer's disease, Gilles de la Tourette's disease, and senile dementia; or behavior disorders where there are strong indications of an organic pathophysiology. Patients with tardive dyskinesia will also be studied. Data obtained from the studies in patients will be of value not only in providing a better understanding of the movement disorders which form a part of the syndromes to be investigated, but also as a means of assessing the effectiveness of drug treatments currently used in patients. Normal subjects will be employed in these studies to provide comparative data for healthy individuals carrying out these tests of psychomotor function.

Publications:

Colburn, T.R. and Evarts, E.V.: Use of brushless DC torque motors in studies of neuromuscular function. In Desmedt, J.E. (Ed.): Cerebral Motor Control in Man: Long Loop Mechanisms, Progress in Clinical Neurophysiology, vol. 4. Basel, Karger, 1978, pp. 153-166.

Evarts, E.V. and Vaughn, W.J.: Intended arm movements in response to externally produced arm displacements in man. In Desmedt, J.E. (Ed.): Cerebral Motor Control in Man: Long Loop Mechanisms, Progress in Clinical Neurophysiology, vol. 4. Basel, Karger, 1978, pp. 178-192.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 01335-08 SMRC

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Studies of Schizophrenia

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
8.2	4.2	4.0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The project summarizes the extensive studies with chronic schizophrenics including the development of a rating scale for measuring change over time the therapeutic testing of 5-hydroxytryptophan, tryptophan, alpha-methyl-para-tyrosine, pineal extract, physostigmine, lithium, baclofen, thyrotropin releasing hormone, naloxene, carbidopa, propanolol, as well as many biochemical determinations including monoamine oxidase.

Project Description:

Objectives:

To test existing and create new hypotheses relating to the possible organic basis of schizophrenia. This project attempts to synthesize psychological, physiological, and biochemical disciplines around the subject of schizophrenia.

Methods Employed:

All studies have been carried out in phenothiazine refractory chronic schizophrenics. The drugs were given and measurements made in a double-blind fashion.

Major Findings:

I. The Rating Scales

Nurses' Rating: Experienced gained with the rating scale we had been using on the research wards to record clinical status indicated that it had many short-comings. We, therefore, undertook to design a more useful nursing scale that could yield quantitative estimates of the level of psychopathology of chronic schizophrenics. To be useful, the scale had to be objective as possible and be comprehensible to nursing staff trained to the level of nursing assistant.

Numerous conferences were held with the nursing staff to determine which psychiatric concepts were comprehended and could be inquired about on a rating form. Sample forms were devised and tested in the following manner: Every staff member on each shift would complete the rating on two patients. The results of this exercise were then subjected to statistical analysis in order to determine reliability and to find if, in fact, any meaningful consensus had been found as to the meaning of the questions. Different pairs of patients were used and the same patients were tested again to determine if reliability was consistent over time.

We have evolved a rating scale that appears usable by nursing staff trained to the nursing assistant level which will accurately reflect the daily clinical status of the patients. The new nurses' rating scale was tested extensively and various ambiguities eliminated. In an effort to shorten the scale correlations were run between specific questions, these correlations did not hold consistently in each patient. Thus, no questions could be demonstrated to be expendable. Computor programs for processing the scale have been devised and a new optical scanning machine activated so that quick feedback can be gained from each patient.

The Inpatient Behavioral Rating Scale (IBRS) is an inventory designed to record daily nursing observations of mood and behavior of patients in a clini-

cal setting. It consists of 26 items characterizing a broad range of psychopathology and is particularly suitable for use where change in mood and behavior is relevant to clinical investigation. It is formated on an optical scan sheet for ease of computer entry and analysis. In over a year of use on two clinical research wards, the IBRS has been found discriminating, reliable, and easy to use by the nursing staff.

We have also contributed materially to the development of a new nurses' rating scale proposed for use throughout the Bethesda and Saint Elizabeths' wards of NIMH. This scale has been keyed particularly to the well established BPRS of Overall and Gorham, and if it can be successfully taught and completed by nursing personnel, will considerably enhance the process of communication of our findings to the psychiatric professional community. This effort is part of a larger effort at standardizing to some extent the procedures and forms used on the clinical units in Bethesda and at Saint Elizabeths Hospital.

Pre-Morbid Rating Scale: During the past six to eight months we have put together a rating scale aimed at assessing pre-morbid adjustment in chronic schizophrenic patients. Items in the scale were either original or adopted and modified items from the Phillips, Gittelman-Klein and Elgin scales. A group of approximately 15 normals (staff of LCP) provided self ratings. To date, approximately 20 chronic schizophrenics from the William A. White Building have been rated from chart material. A total of 44 chronic schizophrenics from Saint Elizabeths Hospital will be rated, along with a larger group of normals. Dr. Phillip Berger is presently using the scale at a VA hospital to provide information on variations in hospital populations and raters. When the scale is eventually able to differentiate subgroups of patients, it will be used in conjunction with the ongoing biochemical studies of chronic schizophrenia.

Wechsler Adult Intelligence Scale: A previous study has shown that parkinsonian patients treated with L-DOPA had decreased scores over time on the Comprehension subtest of the Wechsler Adult Intelligence Scale (WAIS), whereas scores on all other 10 subtests of the WAIS increased. It was hypothesized that if L-DOPA treatment, which increases dopamine activity in the brain, is directly related to an apparent deleterious effect on the WAIS Comprehension, then a drug such as alpha-methyl-para-tyrozine (AMPT), which decreases dopamine activity, might have an augmenting effect on this subtest. A therapeutic trial of AMPT in a group of chronic schizophrenic patients provided an opportunity to test this hypothesis. It was found that Comprehension scores improved significantly with AMPT. Other clinical rating instruments failed to show any changes. The implications of using a psychometric instrument to assess specific, but clinically obscured, drug effects on intellectual functioning are discussed.

Scores from 94 psychiatric and neurological patients on the 11 subtests of the WAIS and the Halstead-Reitan Category and Finger Tapping tests were used in a factor analysis. The Category Test had a low loading on the first

verbal, factor and a highly loading on the second, visuomotor factor; the Tapping Test had a high loading on a small fourth factor of manipulative speed. The finding with the Category Test suggests that it, contrary to the expectation of many of its users, does not distinguish an ability that is both separate from nonverbal intelligence and particularly sensitive to all types of brain pathology. It is concluded that visuomotor and verbal factor scores might be more efficient than conventional scores in studies of effects of brain damage.

II. Serotonin and Its Precursors

A. Behavioral: The study has now been underway for about five years. The first three patients were given alpha-methyl-5-hydroxytrytophan for one month preceded and followed by one month placebo periods. The active drug made these patients worse.

The subsequent 18 patients were given L-5-hydroxytryptophan together with a peripheral decarboxylase inhibitor (MK-486). MK-486 blocks the conversion of 5HTP to serotonin outside the brain and because MK-486 does not enter the brain makes more 5HTP available for conversion to serotonin within the brain parenchyma. In addition, it is thought that MK-486 reduces 5HTP's side effects.

The patients were given placebo for one month, the active drugs for up to three months, and then a second placebo for one month. Behavioral ratings were done by nurses four times daily. The last 7 days of the highest active drug period were compared to the last 7 days of both placebo periods. For a change to have taken place, we required that the active drug period had to be higher or lower than both placebo periods ($p<0.05$, 2 tailed "t" test). By the criteria indicated, 3 patients have improved, 3 have become worse, and for 12, there was no change. It must be emphasized that these comparisons were made against placebo only and as yet, we have not determined how 5HTP compares to conventional drugs. What is clear is that these drug combinations chemically differentiate at least two kinds of schizophrenic patients; those who become better and those who become worse. Further efforts to determine if these chemical differentiations correlate with any behavioral distinctions are being made.

In order to determine if there are any additive effects of 5HTP and neuroleptics, we are now treating our patients with a constant dose of a neuroleptic, MK-486 and 5HTP (3-6 mg/day). The first three patients were given chlorpromazine as the neuroleptic and appeared to do well. The neuroleptic was then changed to haloperidol. Five male and two female chronic schizophrenics received a combination of haloperidol, L-5HTP and MK-486 in a double-blind, crossover study.

Patients were stabilized for at least one month on an optimal dose of haloperidol and an antiparkinson agent when necessary. After a month on haloperidol and placebo -5HPT, patients began L-5HTP, 100 mg/24 hours, along with MK-486, 200 mg/24 hours. The intent was to follow a program of stepwise

increments of L-5HTP dosage to achieve levels of 3 or more grams/24 hours as in the previous study, but this turned out to be impossible. Of the 7 patients, 3 became so activated on 100 mg/24 hrs of L-5HTP that they had to be removed from the protocol. All 7 patients showed some activation of illness in terms of their underlying pathology or character traits, but in 4 of the 7, these particular reactions subsided quickly, and permitted increased dosage of L-5HTP.

Preliminary data suggest that with each increment of L-5HTP, there was an increment of agitation or an exaggeration of existing pathology. There then followed in some patients an improved clinical status with better integration of thinking and more social interaction. This kind of improvement was vividly reported by 3 patients' families, who were, of course, unaware of the timing of the medication change.

These measurable clinical changes are striking, considering that such low doses of L-5HTP were used. We found earlier that without haloperidol, activation of symptoms occurred only when doses of L-5HTP of 2 to 3 grams per 24 hrs were reached. Tolerance to some side effects of L-5HTP developed in the patients during both studies. Transient parkinsonian side effects occurred in this study, whereas they did not at the much higher levels of 5HTP given in absence of haloperidol. It is clear that haloperidol in some way sensitized the central nervous system to exogenous L-5HTP.

Nine new chronic schizophrenic patients, three females and six males, participated in a second study of the clinical effectiveness of the drug combination 5HTP, chlorpromazine and MK-486. Doses up to 4 gm daily of 5HTP were given along with variable amounts of chlorpromazine. A common daily dose of chlorpromazine was 800 mg; however, patients received 200 mg MK-486/24 hrs. Although daily ratings of patients' attitudes and behavior are not yet available for analysis, no sustained clinical improvement was obvious in any of the patients. A preliminary impression is that 5HTP combined with chlorpromazine, like 5HTP and haloperidol, does elicit an increase in expression of affect. One patient in particular said repeatedly during active drug periods that she felt better, but no lasting change in paranoid ideas was seen. Apparently large doses of 5HTP in combination does not add much to therapy and may produce undesireable displays of irritability or uncontrolled affect in some chronic schizophrenic patients. An occasional patient may experience pleasurable affect on 5HTP without other marked change in clinical state.

B. Sleep Studies: Previously we found that chronic schizophrenic patients had a decreased number of rapid eye movements during REM sleep (REM density) and an increased number in NREM sleep. This situation is similar to one seen after the administration of parachlorophenylalanine (PCPA). PCPA decreases serotonin by inhibiting tryptophan hydroxylase. 5HTP reverses the sleep effects of PCPA-treated patients.

The sleep of 18 male, chronically hospitalized, schizophrenic patients was studied during prolonged 5HTP administration. This was compared to sleep studies of various lengths during the month-long placebo periods and immediately preceding and following 5HTP ingestion. During active drug administration, the peripheral decarboxylase inhibitor, MK-486, was also given. Seven of the 18 patients were studied at intermediate doses, 11 at high doses, and 7 during withdrawal from 5HTP.

EFFECT OF 5HTP ADMINISTRATION AND WITHDRAWAL ON MEAN SLEEP PARAMETERS

	P_1	5HTP (3600-5500mg)	P_1	5HTP (5600-6000mg)	P_1	Withdrawal
No. Subjects		7		11		7
Total Sleep	318	274	335	273***	297	213
Stage II	223	209	219	18*	190	195**
Delta	6	0.04**	19	6***	28	11**
REM Latency	71	192	70	175***	91	19*
REM Time	78	52	86	69***	71	77
REM Density (Index)	1.4	1.6	1.5	1.6	1.5	1.7
REM %	23.2	19.4	24.3	25.2	21.9	36.2*

P1- Sleep during the month placebo immediately preceding drug
Withdrawal - Sleep during the first week following last drug administration
*p<.05 (Wilson matched-paired signed rank test)
**p<.02
***p<.01
Values are in minutes/night except for REM % and REM Density

At low dosages of 5HTP (<2000mg) there was a significant increase in REM Latency (190 vs. 65), but no other changes in sleep. Total REM Sleep and Delta were significantly reduced on high dosages. Patients were agitated and sleep was poor during the first week of withdrawal; however, there was a significant increase in REM % and a decrease in REM Latency. During the second week of 5HTP withdrawal, all sleep measurements returned to baseline.

In addition, at the high dosages, the number of REM periods decreased compared to placebo (2.1 vs. 3.7, p<.01). Although the length of the first REM period was increased on high 5HTP dosages (43 vs. 18 min, p<.02), the time from onset of the first REM period to the second was unaltered.

The interpretation of the effects of 5HTP on sleep are complicated by such factors as dose, tolerance, and patient's behavioral response. Contrary to the hypothesis relating serotonin to sleep maintenance, total sleep decreased on 5HTP and contrary to the phasic-event suppression hypothesis, the REM density was not affected; however, REM density may not be the appropriate measurement of PGO spike density.

C. Biochemical: Cholera toxin is a non-reversible activator of adenylate cyclase from several tissues. Since adenylate cyclase is thought to be part of the receptor-complex in many neurons, we used cholera toxin to study whether adenylate cyclase was linked to regulatory receptors of serotonergic neurons. The rate of 5-HT metabolism was taken as an index of neuronal activation and we measured this metabolism in brain areas of rats injected with cholera toxin into specific brain regions.

Cholera toxin was injected into the raphe nuclei or caudate nucleus and serotonin metabolism was studied by high pressure liquid chromatography.

When cholera toxin was injected into the raphe nuclei, the metabolism of serotonin was increased in the striatal regions on both sides of the brain. Increased metabolism was manifested by an increase in the concentration of 5-hydroxyindoleacetic acid (5HIAA) for more than 72 hours. The change was a direct result of an increased rate of formation of serotonin rather than an inability of the brain to dispose of the acid. Injection of the toxin or the vehicle for the toxin into the brain ventricles was without effect on the serotonin system. Injection of toxin into the caudate nucleus on one side of the brain resulted in increased levels of 5HIAA in the dorsal raphe nucleus and striatum on the opposite side of the brain. There were no detectable changes of 5HIAA in the medial raphe, whose axons do not project to the striatum. Activation of the dorsal raphe was apparently by a retrograde process. The basal levels of adenylate cylcase were enhanced on the injected side of brain; however, there was no change of adenylate cyclase activity on the contralateral side of brain even though 5HIAA levels increased. The rise of 5HIAA on the contralateral side of brain was prevented by a hemisection caudal to the injection site. Our results suggest that it is possible to biochemically activate the metabolism of brain serotonin for days using regional injections of cholera toxin. Activation is not necessarily associated with long term changes of adenylate cyclase activity. Moreover, it appears that both caudate nuclei may be innervated by a single cell body or by separate cell bodies in ready communication.

Many psychiatric disorders are associated with abnormal metabolism of the biogenic amines in brain. Adenylate cyclase can be activated by the biogenic amines. Cholera toxin offers the opportunity for understanding the mechanisms for activating the cyclases of the amine containing neurons for prolonged periods.

The Habenula: Anatomical evidence suggests afferents to the raphe nuclei originating in the lateral habenula. The habenula nuclei have been proposed to play an unique role in funneling information from forebrain structures to the raphe. Supporting this hypothesis, recent electrophysiological experiments characterized neurons in this pathway as inhibiting the firing of raphe neurons. GABA has been postulated as the inhibitory transmitter. We have investigated in rats the short and long-term effects of habenula lesions on serotonin (5HT) turnover in the dorsal raphe and medial raphe and caudate nucleus, a dorsal raphe terminal area. In addition, GABA concentrations were determined in the dorsal raphe. Alteration in 5HIAA levels

following lesions were used to estimate change in 5HT turnover. Both unilateral and bilateral habenula lesions produced statistically significant elevations in 5HIAA in the dorsal raphe when measured 16 hours and 7 days postlesion. There was no change in medial raphe or caudate nucleus 5HIAA. Dorsal raphe GABA concentration did not differ from control 7 days following biolateral habenula lesions. Although anatomical evidence suggests habenular projections to both dorsal and medial raphe, habenular lesions affect 5HT turnover only in the dorsal raphe. The lack of effect of habenular lesions on caudate nucleus 5HT turnover suggests that changes in cell body 5HT metabolism can occur without concomitant changes in terminal projections. These results support the presence of an inhibitory habenular raphe pathway but do not confirm GABA as the inhibitory transmitter.

III. The Catecholamines

A. Behavioral

AMPT (Alpha-Methyl-Para-Tyrosine): Several lines of evidence suggest that certain types of schizophrenia may be associated with excessive dopamine activity. Psychotic symptoms in schizophrenics may be intensified by methylphenidate, which increases functional dopamine activity, and by L-DOPA, which is converted to dopamine. Amphetamine-induced psychosis in volunteers and in "speed freaks" is thought to be mediated by dopamine. The clinical efficacies of neuroleptics probably depend upon blockade of dopamine receptors. Alpha-methyl-para-tyrosine (AMPT) inhibits tyrosine hydroxylase, the rate limiting enzymatic step in dopamine synthesis. Although it should theoretically be clinically helpful in schizophrenics, two previous studies reported no effect when given alone. In a third study, however, Carlsson and his associates found that during treatment with AMPT, less thioridazine was required to treat schizophrenics. In this study, we report results of administering AMPT in a double-blind fashion to chronic schizophrenics who were concurrently treated with high doses of phenothiazines.

The subjects were 10 male chronic schizophrenics (age 19-35) who had been hospitalized for 2-15 years. Each received a constant dose of phenothiazines (average equivalent dose of chlorpromazine=800 mg/day). A four-week period of placebo administration preceded and followed by a three-week trial of AMPT (3 gm/day). Clinical effects were monitored by (a) the NIMH Nursing Rating Scale; (b) the BPRS administered by two psychiatrists weekly; and (c) the Comprehension Subscale of the WAIS before, during, and after AMPT administration.

As shown in the table, significant improvement was observed on the Comprehension Subscale ($p<.001$ compared with placebo periods) on AMPT. However, no improvement was noted in the schizophrenia items of the BPRS or the NIMH Nursing Rating Scale, except for one patient. Clinical impressions of three, nonblind psychiatrists suggested moderate but transient improvement during the first week of AMPT administration in 8 of 10 patients. Two patients developed symptoms on AMPT and one was withdrawn from the study. AMPT's effect on plasma levels of chlorpromazine (measured twice weekly) are presented in the table.

MEAN PLASMA CPZ (ng/nl)		
Placebo	77.7	
AMPT	72.9	
Placebo	109.0	$p<0.01$

AMPT markedly increased prolactin secretion (see table).

MEAN PLASMA PROLACTIN (ng/ml)		
Placebo	46.1	
AMPT	111.6	$p<0.002$
Placebo	42.4	$p<0.01$

MEAN (±SEM) SCORES ON FIVE CLINICAL OR PSYCHOLOGICAL VARIATIONS WITH AMPT

	Placebo	AMPT	Placebo	Analysis of Variance
Mean Psychosis Score (NIMH Inpatient Behavioral Rating Scale)	11.4±1.1	11.9±0.9	1.2±1.4	$X^2_r = 4.20$, NS
Mean Schizophrenia Score (BPRS)	9.5±1.2	7.5±1.1	8.4±1.1	$X^2_r = 5.85$, NS
Mean Comprehension Subtest Score (WIAS)	6.6±0.9	10.3±0.8	8.0±0.8	$X^2_r = 11.56$ ($p<0.001$)
Mean Depression Score (NIMH Inpatient Behavioral Rating Scale)	7.0±0.5	8.0±0.4	7.1±0.5	$X^2_r = 5.45$, NS
Mean Depression Score (BPRS)	5.9 1.3	4.8 1.2	4.8 1.3	$X^2_r = 0.95$, NS

The results suggest that during a three-week trial with AMPT, an inhibitor of dopamine synthesis, subtle or no improvement in psychological functioning (judgment and comprehension) occurred in chronic schizophrenic patients on phenothiazines. Significant clinical improvement was not detected on the

BPRS and NIMH Nursing Rating Scale.

In a second study, we administered AMPT (3 gm/day) for two-three months to seven chronic schizophrenic patients who were treated with thioridazine (800 mg/day). No major clinical benefits were documented in the second study, although serum prolactin appears to have been elevated, an indication of central dopaminergic blockade.

7-Hydroxy-Chlorpromazine: A number of biochemical, animal and human studies suggest that 7-hydroxy-chlorpromazine (7-OH CPZ) is an active metabolite of chlorpromazine (CPZ). This hypothesis was tested by administering 7-OH CPZ to four hospitalized chronic schizophrenic males (Research Diagnostic Criteria) using a double-blind crossover design.

Following a four-week placebo period, 7-OH CPZ was given for at least four weeks, three weeks of which were at 1000 mg/day. A second four-week placebo period was then followed by the administration of CPZ at the same dose and duration as 7-OH CPZ. Throughout the study, patients were evaluated twice/day with a nurses' rating scale and once/week with the BPRS. Serum prolactin levels were measured at least once/week. Data was evaluated by an analysis of variance and the Newman-Keul test.

Patients improved on 7-OH CPZ relative to placebo on the following NRS items: tension and uncooperativeness ($p<.01$); and blunted affect, hostility, excitement and arousal ($p<.05$). With regard to CPZ relative to placebo, improvement was demonstrated on tension and blunted affect on the NRS ($p<.05$). 7-OH CPZ was more effective than CPZ on uncooperativeness on the NRS ($p<.01$). On the BPRS, patients improved 7-OH CPZ relative to placebo on total score and emotional withdrawal ($p<.05$). Again, on the BPRS, CPZ was more effective than placebo on activation and anxiety-depression ($p<.05$). Finally, 7OH CPZ reduced emotional withdrawal more than CPZ ($p<.05$) as measured on the BPRS. Improvements on the NRS and the BPRS ranged from 25.5% to 50.9% for statistically significant items. Prolactin levels on 7-OH CPZ and CPZ were 33.1±4.5 ng/ml and 31.1±8.7 ng/ml, respectively. These were elevated by 119.2% and 84.0% over placebo levels of 15.1±4.5 ng/ml and 16.9±4.5 ng/ml, respectively ($p<.05$).

The above results clearly show that 7-OH CPZ is an active neuroleptic producing clinical improvement in chronic male schizophrenics. Moreoever, it caused substantial increases in serum prolactin levels, a property of its metabolic precursor, CPZ, and numerous other antipsychotic dopamine blocking neuroleptics.

Propanolol: We have initiated a pilot study evaluating the therapeutic potential of high doses of β-adrenergic blocking agent propanolol in chronic schizophrenia. One patient has completed the study and showed no clear diminuition of her psychosis. Preliminary data indicates that there may have been a paradoxical increase in the number of available B-receptors in this patients lymphocytes, associated with the high doses of the B-blocking agent. Other patients are in the protocol currently.

IV. Physostigmine

Ten patients have received oral physostigmine in varying degree in a controlled double-blind protocol attempting to replicate the earlier findings of Rosenthal that brief but dramatic clinical improvement could be observed during such treatment. None of the patients studied showed significant improvement. A report of this finding is in preparation. No further studies are contemplated.

V. Pineal Extract

Dr. Altschule reported in 1957 that injections of an aqueous extract of bovine pineal substance exerted a beneficial effect in chronic schizophrenia. A subsequent blind study on eight elderly, extremely chronic lobotomized schizophrenic women failed to show major therapeutic potential of the extract. We are testing the extract on a less chronically-ill population to see if there might be any merit to Altschule's earlier observations.

When the patients are clinically stable they are started blind on either a course of placebo or pineal extract. After 4-6 weeks on this medication, there is a crossover to the type material not already received. If the findings warrant, there is another shift of medication.

From the previous study done here using younger patients, it appears that there is a statistically significant but generally slight beneficial effect exerted by the extract on some patients. Only one patient can be said to have achieved a major remission associated with extract treatment. This finding has been confirmed in numerous crossover experiments.

A further three patients have been tried on pineal extract. Of the three one responded with significant clinical improvement. Six months after completing the protocol, this patient was given a second trial of pineal extract vs. placebo injections and again showed significant clinical improvement coincident with extract administration.

The most pronounced responder had been tried on enteric coated pineal tablets. Although an open study, the results were sufficiently encouraging to justify having more of these tablets made for further clinical trials, again in selected patients. The advantage of administering the pineal substance in this form is that dosage can be increased. The earlier studies with intramuscular preparations was handicapped by the inherent volume limitations imposed by that route of administration.

Two additional patients have been tried on enteric coated pineal powder tablets. One became markedly aroused and hostile; with the other there was no change. The one patient already shown experimentally to be a marked responder to the aqueous extract and thought in an open study to be responsive to the oral preparation went through a double-blind placebo substitution. The patient showed worsening of her psychosis (as judged by the nursing ratings) while on

placebo and improvement when active medication was substituted. Further extensive clinical testing of pineal materials does not seem justified at this time. Efforts are being made using experimental animals to develop techniques for either predicting who the few pineal responsive patients might be or what might be a possible mechanism of action. Until a working hypothesis along these lines can be developed, it seems more practical to test clinically only those few patients who have a clinical profile quite similar to that of the most responsive patient.

We were unable to isolate a fraction of the pineal extract that would be significantly enriched with regard to inhibitory activity on the in vitro assay of indoleethylamine-N-methyl transferase. Further study with pineal and liver extracts indicated that the inhibition obtained from extracts of these tissues was quite non-specific.

VI. Lithium Carbonate with Other Neuroleptic Drugs

There is good documentation in the literature indicating that chlorpromazine is superior to lithium in acute schizophrenic illness. The superiority of chlorpromazine in controlling extreme mania also appears to be established. Publications bearing on the use of lithium in schizo-affective illness are less unanimous in their conclusions. All of the studies so far reported do not indicate a superiority for the use of lithium over chlorpromazine in patients diagnosed as schizo-affective. There are no studies of the merits of using both medications simultaneously. It is our personal experience that many clinicians are using lithium carbonate in combination with major tranquilizers in the treatment of schizophrenic and schizo-affective patients, despite the fact that there are no controlled experiments in the literature bearing on the efficacy of this combination.

This study has been expanded and is taking place mainly outside the William A. White Research wards. This has been done for a number of reasons, among them our interest in being able to study a greater number of patients on low- risk protocols such as this one. After several month's work, three wards in other services have been trained in the use of the rating instruments. In addition, one of the wards is in John Howard Division. This setting offers an excellent opportunity to study the effects of lithium ion on various aspects of aggression. The protocol has been appropriately modified for this. Four patients have completed the protocol; five more are pending. Preliminary analysis of the data indicated that the clinical indications for combined treatment with lithium and phenothiazines are likely to be complex.

VII. GABA

A. Clinical

Baclofen (Lioresal): In the spring of 1975, a brief clinical report appeared in Lancet stating that 13 treatment resistant chronic schizophrenics

were found to improve and require less conventional neuroleptic medication w.en ... d on Baclofen. Since Baclofen, then thought to be a GABA agonist but now thought to be an antagonist of substance P, is a compound in no way similar to currently used neuroleptic agents we have conducted a clinical trial. The results from the nine patients have been uniformly negative (see table).

SYMPTOM CLUSTER

Psychosis		Arousal		Depression	
Placebo	Baclofen	Placebo	Baclofen	Placebo	Baclofen
14.3	14	11.2	11.7	6.8	7.2

B. Animal

Imidazoleacetic Acid: Chronic administration of neuroleptic drugs to animals results in a syndrome of behavioral hypersensitivity to dopaminergic agonists. It is thought that this is a manifestation of the development of postsynaptic supersensitivity to dopamine, induced by the dopamine-receptor blocking activity of these drugs. The phenomenon of postsynaptic denervation supersensitivity has long been known to be nonspecific in regard to particular neurotransmitters; this would suggest that chronic treatment of animals with dopamine agonists would also induce alterations in behavioral sensitivity to any other neurotransmitters for which receptors are located on the same neurons as dopamine receptors. There are gamma-aminobutyric acid (GABA) receptors in the striatum, and these seem to be initmately associated with dopamine-receptive neurons. It was therefore decided to test the possibility that alterations in the behavioral sensitivity to GABA agonists accompany the behavioral hypersensitivity to dopamine that is induced by chronic treatment with the dopamine-blocking neuroleptic haloperidol. It was found that the ability of the GABA receptor agonist imidazole-4-acetic acid (IMA) to reduce motor activity was lessened in animals that had been given haloperidol chronically added to the drinking water. When the time course of the behavior of the animals were examined, it was found that the activity of the control animals was high when first placed in a novel environment, but habituated gradually over the course of testing. Animals treated chronically with haloperidol, or injected with IMA, showed relatively small changes in total activity but essentially the same pattern of habituation. On the other hand, animals treated chronically with haloperidol and also injected with IMA showed almost a complete absence of habituation, in that their activity remained at or near its initial levels for the enture duration of a two-hour testing session. Acute injections of haloperidol had no such effect, and were simply additive with IMA in terms of decreasing behavioral activity. It is suggested that this alteration in responsivity to IMA, and presumably to central nervous system GABA, plays a role in the therapeutic response of schizophrenic patients to haloperidol.

VIII. Thyrotropin Releasing Hormone In Chronic Schizophrenia

Intravenous administration of synthetic thyrotropin releasing hormone (TRH) has been reported to lead to a prompt amelioration of schizophrenic symptoms in a pilot study involving four female patients. Drayson gave TRH to three patients with cyclical psychoses and failed to observe any clinical change. We gave TRH to three treatment resistant chronic schizophrenic males in an inpatient research setting in which all subjects were drug free for at least a month prior to study. Synthetic TRH, 600 μg (Abbott 38579) or matching placebo, was administered by slow intravenous injection at 8 a.m. for five days followed by a two-day holiday and then crossover to the treatment not given. Order of treatment was random; neither the person giving the injection nor any of the clinical staff was aware of the nature of the substance being given. The patient's clinical status was estimated by the nurses' rating scale as well as by clinical observation by the resident psychiatrist. Particular attention was paid to each patient the first day of each series of injections because of the reported rapid onset of clinical change. Statistical comparison of active TRH vs. placebo was by t-test of the means for appropriate questions for the five days of each treatment period. The results are summarized in the Table.

EFFECTS OF SYNTHETIC TRH ADMINISTRATION ON THE SYMPTOMS OF SCHIZOPHRENIA IN THREE CHRONIC SCHIZOPHRENIC PATIENTS. #: t-TEST

Patient # Treatment	Psychosis	Depression	Anxiety	Hallucinations & Delusions
Placebo	3.20±.09	1.53±.16	2.80±.15	1.35±.62
1) TRH	2.84±.25	3.18±.25	3.18±.22	2.20±.50
p	NS	.05	NS	NS
Placebo	3.47±.15	1.55±.30	2.68±.16	4.55±.28
2) TRH	3.23±.13	1.23±.20	3.10±.21	4.23±.38
P	NS	NS	NS	NS
Placebo	3.34±.12	2.28±.12	2.93±.24	3.80±.19
3) TRH	3.56±.14	3.53±.30	3.75±.12	5.43±.37
P	NS	.005	.01	.005

The table shows that two of the three patients showed some worsening of their clinical condition, particularly patient 3. It is especially interesting to note that the depression score for these two patients were significantly greater during the week on TRH as compared to the week on placebo. This finding stands in contrast to earlier reports suggesting that TRH has antidepressant activity, and certainly TRH does not appear to be a useful drug for schizophrenia.

IX. Formation of Tryptolines (Tetrahydro-β-Carbolines) by Human Brain and Platelets

We have examined the products formed from tryptamine, N-methyltryptamine, and 5-hydroxytryptamine by an enzymatic preparation from either human brain or platelets, using 5-MTHF as a cofactor. In each case, we have demonstrated by TLC, co-crystallation, and gas chromatography/mass spectrometry that the reaction involves the addition of a carbon unit to the side-chain nitrogen, with subsequent cyclization to yield a tricyclic, tryptoline derivative. These compounds have been found in plants, but have not previously been associated with mammallian systems. For both enzymatic preparations, the optimal pH is 6.5 and phosphate buffers of increasing strength enhance the reaction. For the brain enzyme, methylcobalamine, flavin adenine dinucleotide, and ascorbic acid each enhance the reaction, while cupric ion inhibits it. We have also done preliminary investigations on platelet enzyme activities in normal volunteers. We found marked diurnal changes and wide variability among individuals; however, male enzymatic activities were significantly lower than those for females. These studies demonstrate that 5-MTHF does not act as a methyl donor, as previously believed.

We have examined the properties and distribution of the enzymatic reaction in the human brain, using 5-MTHF as cofactor. With indoleamines, activity increases with either hydroxy or methyl substitution at the 5-position of the aromatic ring and decreases with the addition of methyl groups to the side-chain nitrogen. Both tryptopline and 5-hydroxytryptoline are substrates but 1-methyltryptoline is not. The enzyme has a similar Km for tryptamine and N-methyltryptamine and is inhibited by Cu^{++}, Ca^{++} and dithiothreitol. The enzymatic activity is unevenly distributed in the brain, with the highest activity in the hypothalamus and the lowest activity in the thalamus.

Acute ip injections of 5-hydroxytryptoline (5-HTLN) killed mice and rats between 12 and 48 hours post-injection with an LD_{50} dose of 320 to 350 mg/kg. Two weeks of daily injections of 200 mg/kg or 100 mg/kg 5-HTLN resulted in respective mortalities of 100% and 50%; surviving mice showed no changes in weight or gross appearance of brain, liver, lung, heart, spleen, kidney or adrenal. Neither acute nor chronic injections affected body weight or temperature except immediately before death where both dropped markedly.

In mice, one hour pretreatment with 200 mg/kg 5-HTLN ip, increased secobarbitol-induced "sleep time" (50 mg/kg, ip). At these doses, neither drug, alone, is lethal; yet, 50% of the animals treated with both drugs died 24-36 hours later. Intraventricularly, 10 µg of 5HTLN to rats decreased motor activity for 25 minutes following the injection.

Acute application of 150-300 µg of 5-HTLN to the isolated rat fundus strip contracts the muscle in a manner similar to 2-4 ng of 5-HT. When added to the superfusion medium, 1.0 µg of 5-HTLN causes a tonic increase in muscle tone without blocking 5-HT-induced contractions. Methysergide blocks these

5-HTLN effects. Preliminary studies suggest that 5-HTLN may act on serotonergic mechanism in the brain.

A case of folate-responsive psychosis which was associated with a defect in $N^{5,10}$-methylenetetrahydrofolate reductase (methylene reductase) suggested the need to examine whether abnormally low activity of this enzyme might be of etiological significance in schizophrenia. We found that there was no significant difference in the platelet methylene reductase activity of chronic schizophrenics, compared to either hospitalized or nonhospitalized, age-matched control subjects (see table). Although it is possible that a larger survey might reveal a subpopulation of schizophrenics who are characterized by abnormal methylene reductase activity, this study suggests that chronic schizophrenia is not generally associated with such changes.

Comparison of Platelet Methylene Reductase Activity in Schizophrenic Patients and Control Subjects

	N	Age (Mean±SEM)	Methylene Reductase Activity (Mean±SEM)*	Range*	r**
Normal Controls	15	28.7±0.94	0.86±0.12	0.15±1.36	-0.31
Hospitalized Controls	16	27.7±1.25	0.72±0.13	0.15±1.36	-0.32
Schizophrenics	18	28.1±1.19	1.17±0.18	0.05±2.28	- .35
All Subjects	49	28.1±0.65	0.93±0.09	0.01±2.28	- .31+

*Expressed as nmole/hr/mg protein; **Correlation of age to methylene reductase activity; +$p<0.05$

X. Sleep

The sleep of 13 drug free chronic male schizophrenics was studied during a one-month trial on chlorpromazine (CPZ) and compared with one-month placebo periods. Recordings were made only after subjects had been on the drug or placebo at least three weeks. Sleep Latency and awake time was significantly less on CPZ while Stage II, Delta Sleep, Delta %, Non-REM sleep, REM activity, and REM Density were significantly increased. There were no significant changes in REM Latency, REM time, REM %, Stage II %, and Non-REM %.

We investigated whether schizophrenics have a normal REM rebound following REM deprivation. In addition, since Cartwright et al. previously suggested that "good REM compensators" tend to be field independent, we related a measure of field independence (the portable rod and frame test) to degree of compensation.

Eight actively ill schizophrenic patients (E) (5F, 3M, mean age = 25) and eight non-psychotic control patients (C) (5F, 3M, mean age = 26) were partially

REM deprived by the awakening method for two nights. Sleep on each of five Recovery nights (R_1-5) was compared with that of Baseline Sleep. All patients were unmedicated except for a single control patient who received chlorpromazine.

Mean Baseline Sleep patterns (min) were similar in E and C patient groups:

	Total Sleep	REM Sleep	REM Latency	REM %	Stage III + IV
E	378	93	72	25.0	37
C	390	99	78	25.5	32

Mean loss of REM was 71.8% for E and 72.8% for C. Controls had to be awakened significantly more frequently than E (35 awakenings compared with 28, $p<.05$).

On R_1 mean change in REM compared with Baseline was +38 min for C and -2 min for E ($p<.01$); on R_2 it was +17 for C and -10 for E ($p<.05$). Mean change in REM during all Five Recovery nights was +14.5 min for C and +1 for E ($p<.05$).

A two-way multivariate analysis of variance--one factor being patient groups, the other being a repeated measure, linear trend analysis--revealed that C had significantly different sleep patterns than did E during Recovery ($p<.03$, $F=2.45$). The major source of difference was in REM sleep recovery ($p<.006$, $F=8.06$) but, surprisingly, Stage III+IV was also a significant contributor ($p<.02$, $F=6.13$). E tended to have elevated Stage III+IV on the first two Recovery nights and low and normal amounts on the last two except on the last Recovery night when Stage III+IV tended to be elevated, though not significantly.

REM rebounds during Recovery were correlated with increased Total Sleep ($r=.52$, $p<.01$). Nevertheless, after removing the effect of change in Total Sleep during Recovery by analysis of co-variance, the controls still had the normal pattern of REM rebound and the actively ill schizophrenic patients did not, i.e., the two groups differed in rate of REM decline over the five Recovery nights ($p<.03$).

Amoung four E and four C patients tested, there was a significant correlation between degree of REM rebound and field independence ($p<.05$, $rho=.79$). Mean change in REM during Recovery was 27 min for three field independent patients and -1.5 min among five field dependent subjects ($p=.036$, Mann-Whitney U-test).

These results suggest that actively ill schizophrenics differ from other psychiatric patients in failing to have a normal REM rebound. This difference was not attributable to differences in age, sex, baseline sleep, anxiety, or change in Total Sleep time during Recovery. REM compensation may also be correlated with field independence, though further research needs to be done to substantiate this finding before any conclusions may be drawn.

XI. Evoked Potentials and EEG

We studied the evoked potentials of 21 schizophrenics and 21 controls. They were matched for age and exposed to a 10-minute sequence of frequent and infrequent auditory stimuli. The amplitude of P_3, a response component of the evoked responses to infrequent stimuli, was much larger in the control group ($p<.0003$). Using a P_3 amplitude of 3.20 μV in the first 2.5 minutes as a dividing point, only 7 of the 42 subjects were misclassified. This amplitude had a significant correlation of 0.48 with the patient's age at first hospitalization. The group P_2 amplitude to frequent stimuli was also much larger in the control group ($p<.003$). This amplitude has a significant correlation of -.41 with medication dosage in terms of equivalent amounts of chlorpromazine. Behavioral scales failed to correlate significantly with evoked response measures. Evoked response latencies did not distinguish the two groups.

A study is underway to develop a means of demonstrating whether or not schizophrenic patients have an unusual lateralization of brain functioning. Visual processing of verbal and non-verbal tasks is being tested to show if patients have recognizable differences from normal subjects. The tasks involve the use of a projection tachiostoscope and are being presented to normal subjects to establish normal patterns of response. Patients are being tested also, but nonconsistent results have developed from limited trials. Should the theory behind this study prove workable, it could be applied to tests of various drug effects, or used to monitor the progress of treatment of patients in addition to providing information about a possible aspect of mental dysfunction.

XII. Brain Dopamine-β-Hydroxylase

Stein and Wise (1971) postulated that there might be a deficit in dopamine-β-hydroxylase (DBH), the enzyme that converts dopamine to norepinephrine, in schizophrenic patients.

Wise, Baden and Stein (1974) tested their hypothesis by collecting the brains of 18 chronic schizophrenics dying in a state hospital and 12 persons without a history of psychiatric illness dying suddenly of heart attacks or accidents. The schizophrenics had decreased DBH activity ranging from 49 in the hippocampus, 58 in the diencephalon and 70 percent in pons-medulla. All these differences were statistically significant at $p<.05$. Because of these dramatic differences in the predicted direction, Wise et al. made a number of attempts to control for artifacts. After considering all these sources of artifacts, the large differences between schizophrenic and normal DBH activity remained.

We attempted to replicate their study. Specimens of autopsied brains from five chronic schizophrenic patients were obtained with the aid of the neuropathologist at Saint Elizabeths Hospital. Brain specimens from four chronic schizophrenics and nine controls were obtained by similar arrangement

with the D.C. Medical Examiner's Office. Information about the subjects was obtained by interviews with family members, and hospital and police records. The control brains were obtained from periods without evidence of a psychiatric history, although one was from an individual with a police record and two were from heavy drinkers. The schizophrenics (7 males; 2 females) had a mean age of 49.2±6.3. Four died suddenly after traumatic suicides (in our experience, suicide is common among chronic schizophrenics), three from cardiac arrests, one from pulmonary aspiration, and one suddenly from pulmonary edema. The controls (all male) had a mean age of 42.3±4.5. Six died suddenly of trauma and three from cardiac arrests.

The periaqueductal pons-mesencephalon, hypothalamus and hippocampus were dissected out at autopsy and immediately placed on dry ice and subsequently stored at -80°C for up to one year. Wise et al. (1974) found DBH activity stable for one year at -15°C. There was no difference in mean storage time for the schizophrenics and controls. All DBH assays were performed by a staff member unaware of whether the samples were from schizophrenics or controls.

There was no significant difference in DBH activity in any area between the two groups. While the differences were not statistically different, DBH activity mean for the schizophrenic groups ranged between 77 and 89 percent of controls. Although seven of nine of our patients were taking phenothiazines at the time of death, Wise et al. (1974) indicate that this is probably not a cause of their DBH differences. Rats who had been given chlorpromazine (20 mg/kg 24 hour) for 12 weeks had a small increase in DBH activity. Although the patients in our study were taking a number of non-neuroleptic drugs, there was significant ($p<0.05$) negative correlations between the daily dosage of chlorpromazine or chlorpromazine equivalent and the DBH activity in the hypothalamus ($r=-.60$) and pons ($r=-.65$). This could mean that in the brains of schizophrenics, neuroleptics do decrease DBH activity, or possibly that there is a negative correlation between clinical pathology (as determined by the need for higher dosages) and DBH activity.

Wise et al. (1974) also attempted to determine the effects of post-mortem intervals on brain DBH activity (after a person dies, it may take several hours for that person to reach the morgue). Simulating this situation rats that were killed and allowed to remain three hours at room temperature prior to assay had DBH activity reduced by 27%. In a similar experiment performed in our Laboratory over a six-hour period, there was 15% decrease in activity. Since Wise et al. (1974) human brain post-mortem intervals were "several hours" and ours a mean of 4.2 for the controls and 3.0 hours for the schizophrenics, this does not seem to be an important factor, except possibly for one control who was not brought to the morgue for 27 hours. Since he had the lowest DBH activity of any of the subjects, the DBH activities were examined for the controls with and without this subject's values. The exclusion of this value did not change the failure to reach statistical significance. Two subjects with death-to-morgue times of seven and 11 hours had DBH activities about their group means. One subject with a 3.5 hour time lag was low. All other subjects had intervals of about one hour.

Wise et al. (1974) also compared the effects on brain DBH activity of the time from which the subject reached the morgue to the time of autopsy. They did this by storage of rat brains at 4°C for up to three days and found a decrease in DBH activity of 18%. Their subjects' brains, however, ranged from one day to eight days at this temperature. Because of this, they matched time intervals for controls and schizophrenics and still found the DBH activity of the schizophrenics lower. Our controls were in the morgue for a mean of 9.7±1.9 hours, while the patients were there for 35.6±7.9 hours, which is statistically significant ($t=2.73$, $p<.02$). Furthermore, there are significant negative correlations between the time in the morgue to autopsy and DBH activity in the hippocampus ($r=-.53$, $p<.05$) and hypothalamus ($r=-.49$, $p<.05$) while that for the pons ($r=-.46$) was not significant. This suggests that the uneven distribution between the two groups in morgue time may be responsible for the small non-significant differences in DBH activity in our samples. Since the Wise, Baden and Stein brains came from two sources, it is possible some of their group differences may be due to different storage techniques.

XIII. Dopamine Sensitive Adenylyl Cylcase

Striatal adenylyl cyclase activity from autopsied human brain is selectively stimulated by low concentrations of dopamine. Under the experimental conditions used, dopamine was about three times more potent than norepinephrine. Histamine and serotonin were ineffective. This stimulation by dopamine was competitively inhibited by haloperidol. There was no difference between nine of the autopsied brains (head of caudate) of the control subjects without a psychiatric history and seven patients with chronic schizophrenia.

IX. Biogenic Amines, Their Precursor Amino Acids and Their Metabolites

The role of biogenic amines in mental illness may include amines other than serotonin and catecholamines. In the present state of our understanding, little information is available on these amines. To bridge this gap, we have undertaken the task to develop comprehensive and sensitive mass fragmentographic methods to measure many of the known biogenic amines, their amino acid precursors and their metabolites.

Our intitial goal is to apply the method developed to measure the concentrations of the various amines and their derivatives in plasma, cerebrospinal fluid (CSF) and brain tissues. After establishing the normal ranges of these substances in biological materials, we hope to carry out several clinical and pharmacological studies in order to probe further the possible role of biogenic amines in mental illness.

The principal technique employed is mass fragmentography. To enhance the specificity of the method, selective gas chromatographic columns are used. These columns were chosen after extensive preliminary trials with various stationary phases and with different lengths of columns.

The biogenic amines, their amino acid precursors and their metabolites are partially separated and concentrated by extraction into organic solvents or by thin layer or column chromatography. They are next dried down, derivatized to their appropriate pentafluoropropionate or trifluoroacetate derivatives and then injected into the GC column.

To correct for variations in processing the samples, internal deuterated isomers of the compounds of interest were snythesized, run through the whole procedure and then used to correct for any inconsistency.

The metabolites derived from tyramine, octopamine, dopamine and norepinephrine were extensively studied in the rat brain, lumbar and ventricular CSF. Mass fragmentography was carried out on the methyl ester/pentafluoroproprionate (ME/PFP) derivatives of the acidic and the PFP derivatives of the alcoholic metabolites, employing an 8 ft. 3% SE 52 column.

In nine patients with ventricular-atrial shunts (5 males, 4 females, age infancy to 65), ventricular metabolite concentrations (ng/ml, mean ± S.E.M.) were: p-hydroxyphenylacetic acid (22.4±3.6), p-hydroxymandelic acid (7.1±1), vanillylmandelic acid (3.1±0.3), dihydroxyphenylacetic acid (9.7±1.6), homovanillic acid (154±14.4), free 3-methoxy-4-hydroxyphenylglycol (MHPG) (11.7±1), conjugated MHPG (4.6±1.3), free p-hydroxyphenylethanol (PHPE) (12.3±1.7) conjugated PHPE (less than 2 ng/ml). The levels of the acidic metabolites in the lumbar CSF were all significantly lower than those in the ventricular CSF. The alcoholic metabolites on the other hand were not significantly different. P-hydroxyphenylglycol was also semi-quantitatively measured and found to be present almost completely in the free form.

Lumbar cerebrospinal fluid (CSF) of schizophrenic patients was studied.

Treatment with oral probenecid (100 mg/kg) increased the concentrations of p-hydroxyphenylacetic, 3,4-dihydroxyphenylacetic and homovanillic acid by over three-folds compared to the baseline levels. The concentration of p-hydroxymandelic acid was also increased, but only moderately compared to the increase of homovanillic acid. Vanilmandelic acid showed a tendency towards being elevated by probenecid, but in our study, the elevation was not statistically significant, probably due to the large variance between subjects.

P-hdyroxyphenylacetic, p-hydroxymandelic, homovanillic, 3,4-dihydroxyphenylacetic, vanilmandelic acids and 3-methoxy-4-hydroxyphenylglycol were measured in control, acutely treated and ethanol-dependent rats.

Ethanol did not cause a shift from an oxidative to a reductive pathway in the metabolism of amines as is well established in the periphery. Ethanol was found to have no effect on the transport of the acidic metabolites across the blood brain barrier.

Metabolites derived from dopamine and norepinephrine were significantly

elevated in both acutely and chronically treated rats while blood ethanol levels were high. During withdrawal (no ethanol was present in blood) only norepinephrine metabolites remained elevated in rats rendered dependent on ethanol.

The concentration of VMA and MHPG in whole rat brain is 11 and 533 pmol/g, respectively. Both compounds were found in all areas of brain studied with VMA, as a percentage of both metabolites, ranging between about 1 and 8 percent. From the decline of the compounds after pargyline, 75 mg/kg ip, we calculated that the rate of formation of VMA is 15 and for MHPG 202 pmol/g/hr. The fractional rate of elimination of VMA and MHPG is 1.4 and 0.38 hr-1, respectively. The rapid rate of loss of VMA suggests that it is transported from brain. However, we were unable to block the elimination of VMA from brain by treatment with probenecid. In contrast, the elimination of MHPG could be blocked by treatment with probenecid. Our study adds support to the notion that MHPG is a major whereas VMA is a minor product of norepinephrine metabolism in brain.

The concentration of the free, glucuronide and the sulfate conjugated forms of 3-methoxy-4-hydroxyphenylglycol (MHPG) were measured in human plasma, cerebrospinal fluid (CSF) and brain by mass fragmentography.

All three forms of MHPG were detected in the media analyzed. Free MHPG was found to be the predominant form in both the brain and the CSF. The sulfate conjugate of MHPG constitutes about 15% of the total MHPG in the CSF while in the brain the percentage varies between 30% in the hypothalamus and cortex and 80% in the substantia nigra.

The concentration of the glucuronide conjugate of MHPG measured in the brain and CSF represents about 5% of the total MHPG concentration.

In the plasma free MHPG and its glucronide and sulfate conjugates are present in about equal concentrations.

The relative concentrations of the three forms of MHPG measured in plasma, CSF and brain were compared with their concentrations in the urine from previously published results. From this comparison the diagnostic significance of each of the three forms of MHPG in the clinical assessment of central norepinephrine metabolism is discussed.

Human plasma concentratons of the major acidic metabolites of m- and p-tyramine, octopamine, dopamine, epinephrine and norepinephrine was measured. The free and conjugated forms of 3-methoxy-4-hydroxyphenylglycol (MHPG) were also measured. The means and ranges of concentrations of the acid metabolites were comparable except for m-hydroxyphenylacetic acid. Free, sulfate, and glucuronide conjugated MHPG were also found in about equal concentrations.

Vanillylmandelic acid (VMA) and 3-methoxy-4-hydroxyphenylglycol (MHPG) were measured in rat brain. The concentration of VMA and MPHG in whole brain

is 11 and 533 pmol/g, respectively. Both compounds were found in all areas of brain studied with VMA, as a percentage of both metabolites, ranging between about 1 and 8%. From the decline of the compounds after pargyline, 75 mg/kg ip, we calculated that the rate of formation of VMA is 15 and for MHPG 202 pmol/g/hr. The fractional rate of elimination of VMA and MHPG is 1.4 and 0.38 h^{-1}, repsectively. The rapid rate of loss of VMA suggests that it is transported from brain. However, we were unable to block the elimination of VMA from brain by treatment with probenecid. In contrast, the elimination of MHPG could be blocked by treatment with probenecid. Our study adds support to the notion that MHPG is a major whereas VMA is a minor product of norpinephrine metabolism in brain.

In a collaborative study with Dr. Norton Neff of the Laboratory of Preclinical Psychopharmacology, dopamine metabolism was studied in various regions of rat brain following the decline of 3,4-dihydroxyphenylacetic acid (DOPAC) and 3-methoxy-4-hydroxyphenylacetic acid (HVA) from brain after treatment with pargyline, or from the accumulation of the acids after treatment with probenecid. The decline of DOPAC and HVA after pargyline treatment appeared exponential in all regions of brain studied with half-lives of about 13 min for HVA and 6.5 min for DOPAC. DOPAC was the major metabolite of dopamine, with various brain regions producing between 2 to 5 times more DOPAC than HVA. HVA accumulated after treatment with probenecid but the accumulation in one hour did not account for all of the HVA apparently eliminated from brain. DOPAC accumulated in some regions of brain (medulla, hypothalamus, and midbrain) and not in others (cerebellum, cortex, striatum, and hippocampus) after probenecid treatment. We conclude that dopamine metabolism is not uniform in brain and that the accumulation of DOPAC and HVA in brain after probenecid treatment only accounts for a minor action of the dopamine formed in brain.

Also in collaboration with the Laboratory of Preclinical Psychopharmacology a study was undertaken to measure phenylalanine hydroxylase activity *in vivo* by measuring ^{18}O-tyrosine in plasma after exposing rats to ^{18}O. We were able to show that ^{18}O is incorporated in tyrosine *in vivo* and that the presence of ^{18}O-tyrosine in plasma is a relative measure of phenylalanine hydroxylase activity. Treatment with p-chlorophenylalanine decreases the ^{18}O incorporation into tyrosine. Because of the simplicity of the procedure and its easy adaptability to human studies, the incorporation of ^{18}O into endogenous constituents might serve as a useful diagnostic procedure for some metabolic disorders, for example, phenylketonuria. These results also indicate that labelling with ^{18}O cannot be used to measure the turnover rate of brain catecholamines as previously proposed.

Sympathetic Ganglion: We also studied dopamine metabolism and function in the rat superior cervical ganglion. Trans-synaptic modulation of dopamine metabolism in rat superior cervical ganglion was evaluated by contrasting normal and surgically decentralized ganglions. Dopamine metabolism was assessed from the decline of DOPAC after administering pargyline and from the concentration of dopamine and DOPAC after treatment with drugs. The concentrations of

dopamine, HVA and DOPAC were about 19, 2.7 and 51 pmol/ganglion, respectively. DOPAC was the major metabolite of dopamine and its calculated rate of formation was 5.3 pmol/ganglion/min, which is approximately 300 nmol/g/hr. This is about 15 times faster than the rate of formation of dopamine reported for striatum. The rapid formation of dopamine in ganglion was supported by the finding of a rapid decline of dopamine after &-methyltyrosine treatment. In the same rat decentralization of the ganglion reduced the rate of formation of DOPAC, but increased DOPAC formation above normal in the intact sham decentralized ganglion suggesting that a feedback system may modulate dopamine metabolism. Treatment with carbachol alone or in combination with atropine of hexamethonium revealed that dopamine metabolism is modulated by nicotinic and muscarinic mechanisms. HVA and DOPAC accumulated in ganglion after treatment with probenecid indicating that an acid transport system is probably responsible for removing these materials from the ganglion. The similarities between the dopaminergic system of the ganglion and of the brain and the rapid rate of dopamine metabolism in the ganglion supports the notion that dopamine might serve as a transmitter or modulator of synaptic events with the ganglion.

During the last 12 months, our work on the sympathetic ganglion was aimed towards achieving two goals: The first was to gain more knowledge on how preganglionic muscarinic and nicotinic cholinergic imputs affect the small intensely fluorescence (SIF) cells with regard to DA turnover and metabolism. The second goal was to characterize pharmacologically the catecholamine receptors present in the sympathetic ganglion and to determine how these receptors regulate catecholamine turnover and synthesis. The ganglion studies were: superior cervical (SCG) and celiac (CG).

The SIF cells were found to be mostly stimulated by muscarinic preganglionic nerves. For example, decentralization lowers DA turnover as reflected by a significant decrease in DOPAC concentration in the SCG. Administration of carbacol or oxotremorine (muscarinic agonists) increased DOPAC while atropine decreased DOPAC in both SCG and CG. Hexamethonium or arecholine (nicotinic agonists) have very little effects on DOPAC concentrations. However, nicotinic receptors appear to have some inhibitory effects on the muscarinic stimulation of the SIF cells.

The concentrations of DA, NE and DOPAC in the ganglia were critically evaluated after treatment with the following basic drugs: reserpine, haloperidol, apomorphine and phenoxbenzamine.

Reserpine significantly decreased NE and increased DOPAC, but had no effect on DA concentrations in the symapthetic ganglia.

Apomorphine, contrary to what we expected, had no effect on catecholamine and slightly increased DOPAC (we expected a decrease). Pretreatment with apomorphine, however, antagonizes the effects of reserpine, thus indicating that reserpine and apomorphine exert their influences via a common receptor.

Haloperidol increased DOPAC in the CG but not in the SCG. It had no effect on the NE and DA concentrations. These results suggest some differences between the DA receptors in the SCG and the CG.

Phenoxybenzamine had no effect on DA, NE, or DOPAC in the ganglia.

XV. N,N-Dimethyltryptamine (DMT) and Phenylethylamine (PEA)

A. Behavioral

Two separate drug-induced behaviors in the rat that satisfy criteria which must be met for animal models of schizophrenia have been described. N,N-Dimethyltryptamine (DMT) and β-Phenylethylamine (PEA) were the two drugs studied because evidence suggests that they are viable candidates in the pathogenesis of schizophrenia. Both are endogenous compounds, found in human tissue. Both may be differentially elevated in psychiatric patients. When given to man, DMT produces psychological disturbance and amphetamine (almost structually identical to PEA) can produce a syndrome resembling paranoid psychosis. Instead of trying to find an animal equivalent of schizophrenia, we studied the behavioral effects of these compounds in rats and applied to these behaviors criteria derived from the clinical condition of schizophrenia. Both DMT and PEA produced dose response disruption of a well-trained conditioned avoidance response in the shuttlebox and PEA, with monoamine oxidase inhibition by pargyline, induced dose response stereotypy, associated with increased motor activity. The criteria we tested were alterations of the drug induced behaviors by acute or chronic neuroleptic pretreatment and tolerance development. Neuroleptics, which are efficacious in the treatment of schizophrenia, reduced DMT disruption and PEA stereotypy in a dose response relationship. In the case of DMT, disruption was attenuated to a degree directly related to length of neuroleptic pretreatment, consistent with long-term antipsychotic efficacy of neuroleptics. Pretreatment with dopamine antagonists and agonists suggests that the mechanism involved in DMT blockade may be through denervation supersensitivity of dopamine receptors. In the case of PEA, attenuation of stereotyped behavior paralleled the clinical potency of neuroleptics on a mg/kg basis and in terms of relative ratios. Complete tolerance did not develop to DMT disruption or PEA stereotypy, consistent with the chronic course of schizophrenia. However, rapid tolerance develops to PEA disruption of shuttlebox if doses subthreshold on PEA induced stereotypy. The drug induced behavioral models reported here are of particular interest because they involve endogenous compounds implicated in the pathogenesis of schizophrenia and there is some degree of pharmacological isomorphism between these models and the clinical condition of schizophrenia.

B. Clinical

We have measured PEA in the 24-hour urine collections in 11 paranoid, 17 nonparanoid and 30 normal controls. Normal 24-hour excretion of PEA is 2 to 15 μg. Urinary PEA was found to be markedly elevated in 22% of chronic schizophrenics. Subjects were judged to be high PEA excretions only if

elevated values were confirmed in two subsequent urine collections, The elevations found in the schizophrenics were two to three times the normal range. Of the six patients with elevated PEA, five were diagnosed as paranoid.

Phenylacetic acid. A rapid, sensitive method for the quantification of free and total PAA in 10 µl of urine by mass fragmentography was developed. The method is very reproducable showing an interclass correlation of 0.97 seg at >.001 on nine samples done three times. Total PAA was determined in 12 and 24-hour urine collections stored at -40°C. All determinations were done in duplicate (410 determinations, interclass correlation .92-.97 on duplicates).

The schizophrenic patients had a significantly lower ($t=2.05$, $p<.05$) urinary PAA than normals (schizophrenics 95.5±15.5 mg, N=24, and normals 146.2±19.7, N=22). Eight patients consistently showed extremely low PAA excretion, and only three normals showed low excretion.

Using the matched samples test, no significant difference in PAA was found between the 12-hour day urine collections (9:00 a.m. - 9:00 p.m.) and the 12-hour night collections in normals ($t=1.0$). However, the day urine collections in 12 patients were significantly lower in PAA than the night collections ($t=2.44$, $p<.05$). No significant difference was found in urine PAA before and after ingestion of 10 ounces of chocolate (0.19 mg PEA) in five normals but headaches were precipitated in three.

C. Animal

Six individually caged juvenile Macaca mulatta (Rhesus monkeys) were studied during two sessions before and four sessions after IM injections of PEA. During each session, two observers who were blind to treatment conditions, counted checking (a stereotyped form of hypervigilance manifested by abrupt changes in visual field) and 27 other discrete categories of behavior. Differences between pre-drug and post-drug behavioral counts were computed for each animal.

Single injections of PEA produced dose-related (25-75 mg/kg) significant increases in checking and lip smacking (another stereotyped behavior) as well as decreases in goal-directed behaviors such as grooming. These effects, which are often accompanied by variable increases in other stereotyped behavior such as rocking, chewing, gnashing and repetitive pacing were maximal at 15 minutes post injection, still substantial at 30 minutes, and mostly dissipated by one hour. These effects are similar to those induced by the chronic administration of amphetamines. Of interest is that unlike amphetamines, consistent tolerance did not invariably develop to the repetitive administration of PEA (75 mg/kg t.i.d.) for four or nine days.

Pretreatment with alpha-methyl-para-tyrosine (AMPT) (125 mg/kg), but not with phenobarbital (25 mg/kg) substantially reduced the characteristic effects of PEA. However, when the same AMPT pretreatment was followed by a subthres-

hold dose of L-DOPA (5 mg/kg) typical PEA effects were restored. This finding suggests that at least some PEA effects require intact dopaminergic mechanisms and that AMPT does not block PEA receptors.

D. Convulsive Properties

Methionine has been found to alter the behavior of animals and to adversely affect the behavior of schizophrenic humans. It has been suggested that these effects are due either to (1) increased availability of methyl groups for the production of methylated amines; or (2) to the accumulation of toxic metabolites of methionine, such as homocysteine and homocysteic acid, which have convulsive properties. If this latter hypothesis is correct, methionine itself would be expected to have the ability to promote convulsions, either alone or when administered to animals that have been "primed" with subconvulsive dosages of other known convulsants.

Initially, the inability of methionine alone to induce convulsions was demonstrated by (1) administrating solutions containing 250, 500, or 1000 mg/kg of methionine (i.p. or s.q., N=243) and observing the animals for four hours; (2) administering suspensions of 1, 2, or 4 mg/kg of methionine i.p. (N=8 per treatment), and observing the animals for four hours; and (3) infusing methionine (50 mg/ml) into mice via the tail vein at varying rates in total dosages of up to 10 gm/kg (N=8). In no case was a convulsion, tremor, or any convulsive-like phenomenon observed in any mouse given methionine alone. Alterations in behavioral activity were observed; the 250 mg/kg dosage caused small increases in behavioral activity, while the larger dosages (500-4000 mg/kg) caused decreases in activity. However, when methionine (1000 mg/kg) was given to animals pretreated with subconvulsive or threshold convulsive doses of homocysteine or pentylenetetrazol, a pronounced enhancement of convulsive responses was observed: homocysteine seemed to be more effective than pentylenetetrazol in this respect. The convulsion-promoting effect of methionine was not shared by other amino acids, including glutamate, glycine, cysteine, tryptophan, and others. On the other hand, ethionine, an analogue of methionine, was found to enhance convulsions about as effectively as methionine. These results show that methionine has convulsion-promoting properties under certain conditions, and support the suggestion that the organicity produced by methionine clinically is due to the accumulation of metabolites such as homocysteine.

Anticonvulsant Properties of Betaine. Betaine has been found to block the induction of convulsions produced by homocysteine; the reason for this effect has been presumed to be that betaine acts as a source of methyl groups for the methylation of homocysteine. This hypothesis would not predict that betaine would have general anticonvulsant properties. A series of experiments was performed to test this possibility. It was found that betaine (380-1540 mg/kg) partially blocked not only those convulsions induced by homocysteine, but also the convulsions induced by electroconvulsive shock and pentylenetetrazol. If the reason for the anticonvulsant activity of betaine is indeed that it reduces the ratio of free homocysteine to free methionine in the body, the present

finding suggests that homocysteine plays a role in the regulation of neuronal excitability under "normal" circumstances; e.g., in the absence of an administration of exogenous homocysteine.

XVI. Low Platelet Monoamine Oxidase Activity and Schizophrenia

The finding that blood platelet monoamine oxidase (MAO) activity is low in some schizophrenic patients has led us and others to inquire as to its significance. As part of this endeavor, there has been a continuous effort to determine if this low enzyme activity was due to one of the large number of artifacts that have plagued the investigation of biological factors related to psychiatric disorders. The enzyme has been assayed in the laboratory of Dr. Dennis Murphy, who, in addition to his interest in the enzyme in schizophrenia, has been using the platelet as a model for synaptosomes.

A. MAO Assay in Blood Platelets

In our initial studies, venous blood was collected in heparin, or more recently, in Becton-Dickinson Company Vacutainer tubes containing ACD (acid-citrate-dextrose), NIH Formula A, and platelets separated and stored according to previously described methods. MAO activity was determined using tryptamine-2-^{14}C HCl (8×10^{-5}M, 8.9 mCi/mM) as substrate and was expressed as nM of tryptamine coverted per mg of platelet protein. The product was indoleacetaldehyde, whose formation was linear with time and enzyme concentration.

During the last several years, our samples were also obtained in the ACD solution, but the MAO activity was determined in platelet rich plasma. The present assays uses benzylamine-^{14}C (4×10^{-4} mCi/mM) as substrate, and is expressed as nM of product per 10^{-8} platelets per hour. Benzylamine is a good substrate for both platelet MAO and an entirely different enzyme occurring in the plasma. Because of this, platelet rich plasma samples with and without the MAO inhibitor parygline are run. Pargyline (2.4×10^{-4}M) completely inhibits the platelet enzyme without affecting the plasma enzyme. The value of sample with the inhibitor is substracted from the sample without--the difference being the platelet activity.

The advantage of the platelet rich plasma assay over the previous one is in not having to determine protein which can easily be contaminated with red and white cells.

B. Stability of Enzyme

Because at times it is not possible to process blood samples or run enzyme assays immediately, it is necessary to know the stability of the enzyme. Comparisons of blood samples processed immediately or kept on ice or at room temperature for five hours revealed no change in activity. Similarly, once the platelet rich plasma is separated, MAO activity remains stable for at least 30 days when kept at -70°C.

C. Comparison of Assays

Pearson correlations were run for 48 normals and 20 chronic schizophrenics between the two assays. The correlation for the normals was .85 while that for the chronic schizophrenic patients was .76. For the combined group the correlation was .83. All our platelet MAO activities are expressed in benzylamine values with a correction (derived from the normals) for values determined using the tryptamine assay.

D. Subjects

Normal bloods were drawn from normal volunteers and Hospital personnel at both the Clinical Center in Bethesda, Maryland and the William A. White Building of Saint Elizabeths Hospital. The bloods from the acute schizophrenics largely came from a research ward at the Clinical Center while those from the chronic schizophrenic patients came from special research wards at the William A. White Building.

E. Age and Sex

Others have reported that females had 10 percent higher platelet MAO activity than males. Our sample females have a slight but consistently higher MAO activity although this difference is statistically significant only for the young adult group.

F. Drugs

Although early experiments with chlorpromazine in intact systems indicated that chlorpromazine had some effects on amine degradation, this seemed to be due to poor amine accessibility to the enzyme because of alterations in the cell membrane. Edwards and Burns (1974) found that the I50 for amitriptyline was 2×10^{-4}M for human platelet MAO in vitro when benzylamine was used as a substrate. The inhibition was competitive.

In studies on patients given neuroleptics, there does not appear to be a consistent difference between when they are on neuroleptics or off, or between patients on neuroleptics and their siblings not receiving drugs.

G. Hormones

One study using only two points found no alteration in platelet MAO activity during the human menstrual cycle. In another study which utilized samples collected three times a week, there were small (23 percent) peak to trough variations in activity in the majority of women. The peak MAO activity occurred during the preovulatory interval and the nadir occurred five to 11 days later. A small number of patients taking prednisone have been seen and have normal MAO activities. Similarly, there does not appear to be any correspondence between plasma thyroxin and triiodothyronine concentrations in psychiatric patients with low and normal MAO activities.

H. Other Factors

The hematocrit, hemoglobin and platelet counts, serum iron, and iron binding capacity were not systematically different in patients with low and normal MAO activity. Other platelet enzyme activities including succinate dehydrogenase, lactate dehydrogenase and cytochrome C reductase were no different in chronic schizophrenic patients with low MAO than they were in controls.

I. Chronic Schizophrenics

To date we have studied platelet MAO activity in 104 male chronic schizophrenic patients. Their mean ±S.E. MAO activity was 7.86±.56 while the value for 348 normal males (ages 18-50) was 11.04±.29. This difference, using a two-tailed "t" test, was significant at $p<.001$. The mean for the 15 female chronic schizophrenics was 7.04±1.06 while that for 332 normal females was 13.29±0.29 ($p<.001$).

The effect of chronic hospitalization on platelet MAO was studied at the Palo Alto VA Hospital where 50 normal controls were compared to 16 patients hospitalized for traumatic accidents to their spines. There was no difference between MAO activity between groups but 39 chronic schizophrenics had decreased platelet MAO activity.

J. Acute vs. Chronic Schizophrenics

To determine whether acute schizophrenics have a reduction in platelet MAO as did the chronic schizophrenics, platelet MAO of 27 acute schizophrenics were studied. The 10 male acute schizophrenics (ages 16-30) had a mean platelet MAO activity of 10.68±.78 which was no different than that of normal males. The 17 female acute patients had a mean of 13.85±1.12 which was no different from that for the normal females.

K. Subgroups of Chronic Schizophrenics

Platelet MAO in two groups of chronic schizophrenic patients (N=35, $\bar{x}$=7.52; N=42, $\bar{x}$=7.86) was significantly lower than in controls (N=680, $\bar{x}$=12.14). Values for those patients diagnosed as paranoid, (N=8, $\bar{x}$=4.47) were significantly lower when compared to non-paranoid schizophrenics (N=27, $\bar{x}$=8.43) in a retrospective study. These findings were confirmed in a separate prospective study of 42 chronic schizophrenics (paranoid and paranoid features N=24, $\bar{x}$=6.40 compared to non-paranoid N=18, $\bar{x}$=9.81). Schildkaut found low platelet monoamine oxidase to be associated with auditory hallucinations and delusions, usually of a persecutory quality. Twelve out of 16 of these low monoamine oxidase patients were designated paranoid. Reduced platelet MAO activity in normal subjects appears to be associated with a greater tendency towards psychopathology and a perceptual behavior study which is similar to that of paranoid schizophrenics. These data could indicate that paranoid schizophrenia may represent a separate disorder from the other schizophrenias. The correlation

between paranoid schizophrenia and low platelet MAO activity suggests some biochemical difference between the different subgroups of schizophrenia.

L. Specificity with Regard to Other Illness

Since the various psychiatric diagnostic classifications are thought to represent different entities, a defect in one should not be seen in the other. The affective illnesses are the group we most diligently contrasted with the schizophrenias.

Sixteen male and 13 female unipolar depressed patients were found to have platelet MAO activity that was no different from our controls while 16 male and 33 female bipolar I depressed patients had reduced activity ($p<.001$). The bipolar patients had a negative skew which was not as large as that of the chronic schizophrenics. Using six units of MAO for the male normals and eight units for the normal females as a point of cut-off, a point under which less than 10 percent of normals fit, 39 percent of male chronic schizophrenics and 60 percent of the female chronic schizophrenics are found (chi square $p<.001$). The bipolar patients also differed from normals ($p<.001$ males; $p<.02$ females). The male bipolar patients were not significantly different from the male chronic schizophrenic patients, but the percentage of female chronic schizophrenics with MAO activity below eight units was greater than the female bipolar I patients. Thus, although bipolar I patients using the arbitrary cut-off have a mean MAO which is low, it is not clear whether they represent a separate group from the chronic schizophrenic patients.

M. Twins Discordant for Schizophrenia

To exclude the possibility that the low platelet MAO activity seen in the chronic schizophrenics was caused by nongenetic factors as opposed to genetic ones, we studied monozygotic twins discordant for schizophrenia. If the low platelet MAO activity is due to some aspect of being schizophrenic, low platelet MAO should only be present in the schizophrenic twin. Thirteen schizophrenic index twins, all of whom had been hospitalized at least once for schizophrenia and had been extensively studied by Dr. William Pollin and his associates, were examined along with their nonschizophrenic co-twins. At the time of the study, one patient resided within a hospital while five were in remission. Four of the patients had had acute forms of schizophrenia and six patients were not receiving any antipsychotic medication. The nonschizophrenic co-twins had never been hospitalized for a behavioral disorder and were functioning well within their families and communities, except for one individual with borderline psychosocial adjustment.

Only two twin pairs were living in the same household and nine of the co-twins were living in different cities. It was, therefore, necessary to obtain and prepare the blood samples at various facilities throughout the country. Because of this, normal control samples were obtained at the same time as those from the twins. They were coded and shipped to the Laboratory. All samples

were batched together before assay. (In all studies samples are run in a manner so that their origins are unknown to all investigators until the final calculations are made.)

The MAO activity of the 23 normal controls was the same as that obtained at that time for previous controls (16.02). The schizophrenic twins (9.88, $p<.005$) and nonschizophrenic twins (12.08, $p<.05$) were significantly lower than the normals, but there was no difference between the twin groups. There was a significant Pearson correlation ($r=.67$, $p<.01$) between the MAO activities in the schizophrenic and nonschizophrenic twins.

There were four pairs of twins whose MAO activities were especially low. The indexes of all four of these twin pairs were chronic schizophrenics while the more acute patients had MAO activities closer to normal. The severity of impairment (based upon number and duration of hospitalizations) was rated on a 5-point scale for the schizophrenic twins by an investigator with no knowledge of the platelet assay. A forced rank order was then made between the numerical ratings, with the highest number given to the most ill patients. Using this order, and comparing it to the MAO activities, a Spearman rank order correlation of -.54 ($p<.05$) was found.

Platelet MAO activity was also examined in nine monozygotic and 10 dizygotic normal twins. The intraclass correlation coefficient was 0.88 for the monozygotic and 0.45 for the dizygotic twins. The correlations were significantly different from one another (Mann Whitney U $p<.001$). Same-sex siblings matched for age and sex were about the same as the dizygotic twins while unrelated pairs were not significantly correlated with each other. These results are similar to those reported by Nies, Robinson, Lamborn and Lampert (1973) for normal monozygotic and dizygotic twins using benzylamine instead of tryptamine as a substrate.

Taken together this data indicates that platelet MAO activity is in a large part determined by genetic factors and that the low platelet activity seen in the chronic schizophrenics is not secondary to being ill, but is genetically related to the liability to be schizophrenic.

N. Family Studies

In order to further study the relationship of low platelet MAO to schizophrenia the blood of first degree nonschizophrenic relatives of index schizophrenics (N=16) was collected. Dividing schizophrenic indexes at their median, the low indexes had a mean of 4.64 and the high indexes had a mean of 10.81. The mean MAO activity for the low index-first degree relative was 8.56. The mean activity for high index-first degree relatives was 11.84. The difference between the means for the low vs. high first degree relatives was significant ($p<.02$; Mann Whitney U). No attempt was made in this small sample to account for sex differences. These differences between nonschizophrenic first degree relatives of high and low schizophrenics is another indication of the genetic nature of platelet MAO activity.

O. Physical-Chemical Studies

In one study, we determined the rate of heat inactivation of the MAO in a group of chronic schizophrenic patients (N=22) and normal subjects (N=13). Aliquots of platelet MAO were subjected to different temperatures between 56 and 62°C for five minutes. Analysis of the data revealed no significant differences in the rate of heat inactivation between the normal and schizophrenic groups confirming an earlier study from this laboratory. However, when the schizophrenic group was divided into paranoid schizophrenic and chronic undifferentiated schizophrenic (CUS) groups, differences became apparent. The MAO in the paranoid group was most resistent to heat while the MAO in the CUS group was the least resistant; the MAO in the normal group was intermediate in its rate of heat inactivation. There was a significant difference ($p<.05$) between the paranoid and nonparanoid (CUS) groups. Neither schizophrenic group differed significantly from the normal group. However, the combination of the normal plus the CUS group different significantly from the paranoid group ($p<.035$).

In a second study, we have attempted to replicate a finding reported by Berrettini and Vogel that an inhibitor of platelet MAO is present in the plasma of schizophrenic patients that have low MAO activity. If their report were correct, it would provide a mechanism that would explain the reduced MAO activity found in schizophrenic patients. However, we have not been able to replicate their results. In fact, in five of six experiments we have found a slight increase in MAO activity when normal platelet MAO is preincubated with plasma from schizophrenic patients (effect of chronic plasma on Km and Vmax, 1.5±0.05 and 22.1±2.46; effect of schizophrenic plasma on Km and Vmax, 1.8±0.28 and 24.9±1.57). We would conclude from our own studies that an inhibitor does not appear to be present in schizophrenic plasma.

In a third study, we are attempting to replicate Dr. Kobes' finding of a reduced Km in the platelet MAO in a group of paranoid patients compared to a group of normal subjects. Although our preliminary findings are in the right direction, certain methodological problems have become apparent and need to be resolved before we can complete the study.

If this finding can be firmly established in the schizophrenic group, we then plan to proceed and to measure the Km in a group of alcoholic patients and in a group of bipolar depressive patients. The overall purpose would be to determine if the decrease of Km in the schizophrenic patients observed by Dr. Kobes is unique to that patient group or is shared by the other two patient groups that have also been reported to have a reduced platelet MAO activity.

P. Relationship of Platelet MAO in Brain MAO

The number of types of MAO's present in the brain is an unresolved question. Original studies indicated that there might be as many as four isoenzymes of MAO within the brain; however, Houslay and Tipton (1973) suggested that the

multiple enzymes found using polyacrylamide-gel electrophoresis might be produced artifactually in a preparatory step. The authors state that these multiple MAO forms may represent a single protein with varying amounts of phospholipid attached; the amount of phospholipid may in turn determine the enzyme mobility during electrophoresis.

Another approach to this problem has come from the finding that selective inhibition of MAO can be used to identify multiple forms of MAO. Clorgyline was the first MAO inhibitor employed in this manner. It was found that tissue homogenates pretreated with increasing concentrations of clorgyline produced stepwise inhibition to the substrate tyramine, suggesting the presence of an enzyme sensitive to the inhibitor and one relatively resistent to it. Considerable more work has been done to identify these enzymes and for convenience they are called Type A and Type B.

The preferred substrates for the Type A MAO are norepinephrine, serotonin, and normetanephrine, while for the Type B they are benzylamine and β-phenylethylamine. Dopamine, tyramine and tryptamine are common substrates. Clorgyline, Lilly 51641, and harmaline are specific inhibitors of Type A while deprenyl and pargyline inhibit Type B. Most of the other MAO inhibitors used in clinical medicine, however, are nonspecific. Platelet MAO, forming one band on electrophoresis, appears to be a single enzyme with Type B characteristics. That is, the platelet enzyme demonstrates only a single sigmoid curve for inhibition using deprenyl, pargyline, or clorgyline. Furthermore, the platelet enzyme is 200-fold more sensitive to inhibition by deprenyl than clorgyline. The conclusion that the platelet enzyme is a simple Type B MAO is also supported by the high activity of the platelet enzyme with the relatively specific MAO-B substrates, benzylamine and β-phenylethylamine, in contrast to its lesser activity with serotonin, an A substrate.

Q. Function

To determine whether or not the low platelet MAO activity might have a functional significance, platelet-rich plasma was obtained from 20 normal controls (age 29.6±1.1) and 16 chronic schizophrenic patients (27±1.4). Since plasma serotonin is almost entirely contained in the platelet the values were expressed in platelet units (ng/10^8 platelets). The platelet counts for the normals and schizophrenics were the same. When the patients had been off phenothiazines (N=12) for 30 days or longer the serotonin concentration was higher (80±4), while when the patients were taking phenothiazines (N=12) their platelet serotonin concentration was normal (90±9). The reason for the discrepancy between patients taking and not taking phenothiazines may be due to the latter's ability to block serotonin uptake into platelets. Nevertheless, since serotonin is not a good substrate for platelet MAO ($K_m > 10^{-3}$) other substrates might be much more affected.

R. Subclass of Schizophrenia

In 1974-1975 we undertook a retrospective study of 43 patients we had examined to look at who the patients were with low platelet MAO. Information was obtained regarding circumstances and age at onset of illness, presenting problems, diagnoses and family history of mental illness. The patient's medical and surgical history was reviewed, as well as laboratory data which included EEG's and skull x-rays. Mental status examination on admission and throughout hospitalization was summarized, as well as changes observed on medication. This information was gathered and coded without knowledge of platelet MAO activity.

Two types of statistical analyses were employed. The first approach correlated MAO activity with a variety of clinical variables using the Pearson product-moment correlation coefficient. The second approach looked for differences between groups of patients with low and high MAO activity. In the second approach, the MAO value of 9.16 units was arbitrarily chosen to divide the chronic schizophrenic patients into high and low MAO groups (12 patients in the high group; 31 patients in the low group). 9.16 is two standard errors above the female chronic schizophrenic mean (8.04) and slightly higher than two standard errors above the male schizophrenic mean (7.86). The term "high MAO" in this study refers to high MAO activity for chronic schizophrenic patients. The high MAO group has a mean ±SEM of 13.54±1.06, which is comparable to values for the normal population. The low MAO group had a mean of 5.47±.37. All patients were diagnosed chronic schizophrenics with a mean hospital stay of 9.3 years, although length of hospitalization within the sample did not correlate with MAO activity ($r=.11$). MAO activity did not correlate with age at onset of symptoms ($r=.06$), age at first hospitalization ($r=.12$), length of hospitalization ($r=.11$), or with problems at work ($r=.20$), school ($r=.07$), or marriage ($r=.12$).

Schizophrenic patients with low MAO had a history of more seizures ($r=.31$, $p<.05$) than patients with high MAO. Of the 41 patients from whom we had an adequate history of seizures, 8 of 30 low MAO schizophrenics had at least one seizure during hospitalization, compared to 1 of 11 high MAO schizophrenics. The high-low difference was not significant using the Fisher Exact Probability Test. None of the patients was on anticonvulsive medication. The possibility of abnormal brain dysfunction correlating with low MAO was reflected in the significant negative correlation between MAO and the number of abnormal EEG's ($r=-.32$, $p<.05$). Of those 36 patients who had EEG records available, 10 of the 25 low MAO schizophrenics had at least one abnormal EEG, compared to none of the 11 high MAO schizophrenics (Fisher Exact Probability Test, $p<.01$). The data indicate that low MAO activity in chronic schizophrenics is associated with an increase in abnormal EEG's and perhaps an increase in seizures. This abnormal brain activity is, however, not associated with other medical or surgical problems, or with any abnormalities in routine blood studies.

In regard to the mental status examination, the patients with low MAO activity were reported to have more ambivalence ($r=-.32$, $p<.05$), but not any

difference in severity or frequency of thought disorder (r=.02), associational defects (r=.10), autistic behavior (r=.03), hallucinations (r=.09), instances of self-mutilation (r=.04) or suicide attempts (r=.24). No evidence for differences in treatment response related to MAO activity was found (r=.05). Mental illness in relatives did not correlate (r=.19) with platelet MAO activity and was not different between the high and low MAO groups. However, it might be impossible to determine such a correlation from the relatively small sample. This is compounded by the inherent difficulties in determining a complete accounting of family incidence of mental illness from chart analysis.

All of the paranoid schizophrenics had low MAO. None of the high MAO activity schizophrenics received a DMS II diagnosis of paranoid schizophrenia during their hospital stay; 7 of the 30 low MAO group did (Fisher Exact Probability Test $p<.05$.) This difference was further substantiated when the means of MAO activity were compared between those who received a diagnosis of chronic paranoid schizophrenia and those who did not. The paranoid schizophrenics have a mean MAO activity of $4.8\pm.46$, which is significantly lower than the nonparanoid schizophrenic values of $8.6\pm.75$ (t-test, $p<.03$). This difference was also reflected in symptoms characteristic of chronic paranoid schizophrenia, such as paranoid delusions and increased hostility, but not in other less specific symptoms.

From this study emerged a picture of a low platelet MAO activity schizophrenic who is more frequently diagnosed as paranoid, as having more paranoid type delusions, and as having abnormal brain activity reflected by increased incidence of seizures and abnormal EEG's.

In order to further examine these findings, 42 new patients were selected on the basis of definite diagnosis of schizophrenia who had active symptoms but without regard to subtype. Patients with a diagnosis of organic brain syndrome or mental retardation or other cerebral pathology, including severe alcoholism, were excluded in order to focus on the paranoid dimensions. The 42 subjects were drawn from a sample of 65. Fifteen were excluded because of their history, eight others met criteria but refused to take part in the study. Seven of these eight refused because of apparent suspiciousness of distrust of the experimental procedure.

All patients met Research Diagnostic Criteria and 41 of 42 met Feighner's criteria for a diagnosis of schizophrenia. The mean age was 35, mean length of hospitalization was 401 months, and all patients had, at one time, received phenothiazine treatment. All patients had a chronic course and 40 of the 42 had a gradual or insidious onset of psychosis. Twenty-nine were males and 13 females. MAO values were 6.7 and 10.6 units for males and females, respectively, compared to mean MAO's of 11.04 and 13.29 for normal males and females. The difference between the schizophrenics and normals was significant at $p<.01$. The clinical staff who best knew the patient rendered a consensus diagnosis based on DSM II categories. A secondary diagnosis was reached for cases of chronic, undifferentiated schizophrenia. All patients underwent a semi-structured interview by research staff which included a shortened version of Wing's Present Exam and a paranoia checklist suggested by Rosenthal.

Interviews were conducted at a time as close as possible to blood drawings, and for most patients were done on one occasions over a 60-mintue period. Repeated interviews were occasionally required to obtain the necessary information.

The information was gathered and coded without knowledge of MAO values. The data indicate that patients diagnosed as paranoid schizophrenics had a mean platelet activity MAO level of 5.4 for males and 8.9 for females, compared ith 8.6 and 12.1 for nonparanoid male and female schizophrenics. Patients with secondary paranoid features had MAO values of 5.6 for males and 9.1 for females. Values for both paranoid patients and patients with secondary paranoid features were significantly different from those of nonparanoid patients ($p<.01$).

The variables which best diagnostically divided the paranoid from nonparanoid patients by a linear discriminate function were: degree of suspiciousness, preoccupation with and systematization of delusions, delusions of control, stereotyped behavior, intact memory, rigidity of ideas, and communication characterized by evasiveness and misleading responses. These diagnostically discriminating items are similar to items on the Venables Scale for Paranoid Schizophrenics except for grandiosity which was not a good discriminator in our study.

These prospective findings confirm the retrospective chart analyses. Chronic schizophrenics had lower platelet MAO activity than normals. Paranoid schizophrenics had an even lower MAO activity level, whether the paranoia was determined by clinical diagnosis, by interview, or by a paranoia dimension scale.

S. Discussion

What does it mean to have low platelet MAO activity in view of seemingly normal brain MAO activity? The argument that post-mortem measurement of brain MAO activity does not reflect its true activity because of autolysis has little support since brain MAO seems to be a very stable enzyme. It, of course, remains a possibility that we have either not measured brain MAO activity in the right region or with correct methods, an argument that is not easily dealt with.

Enzyme activity must now be measured in other regions of the body, such as the muscle, lung, liver, and kidney. If these areas turn out to have normal MAO activity while the platelet is low, intriguing explanations will be needed.

Part of the attractiveness of the concept of low MAO activity is that it is congruent with the two most generative biological hypotheses of schizophrenia. The first of these suggests that there is a functional excess of dopamine. A defect of MAO, an enzyme involved in dopamine metabolism, might be able to produce "dopamine excess." The second hypothesis suggests the formation of an hallucinogenic methylated indoleamine (transmethylation). Since the indolamines

as well as their methylated congeners are metabolized by MAO, this theory is also consistent with a finding of low MAO activity. One of many questions that needs to be answered before low MAO activity can be considered causative, is "Why don't the vast majority of people given drugs which inhibit MAO become psychotic?"

XVII. Naloxone

Naloxone was given in 0.4 mg intravenous doses in a double-blind cross-over design to patients with chronic schizophrenia. Using the BPRS and AIMS as scales, there was no noticeable changes other than an improvement in abnormal thought content.

XVIII. CAT Scan

This study involves quantitative evaluations of computerized tomographic brain scans and quantitative assessments of ventricular volume and cortical sulci size determined from their scans in chronic schizophrenic patients under age 40. This provides a non-invasive, yet accurate means for considering possible abnormalities of these parameters described in prior pneumoencephalographic studies. Preliminary findings suggest that when compared to normal controls, psychiatric patients who do not meet research diagnostic criteria for chronic schizophrenia, and neurological controls, young chronic schizophrenics as a group have larger ventricles and more cortical atrophy. It was also possible that in a small subgroup ventricular enlargement is dramatic. These changes will be investigated to look for possible correlations with treatment variables, past history, neurological abnormalities, etc.

XIX. Calcitonin and Feeding

Plasma calcitonin (CT) concentrations increase following a meal and following the administration of the gastrointestinal hormones glucagon, gastrin, pancreozymin, and cholecystokinin. A series of experiments was performed to test the hypothesis that this food-induced increase in CT secretion plays a role in reducing subsequent food intake. A single S.C. injection of synthetic salmon calcitonin was found to inhibit the 24-hr food intake of rats (up to 58:; $p<0.005$) in a dose-related (12.5-50 U/kg) fashion. This was accompanied by a pronounced diuresis and increase in drinking which were, however, not observed in food-deprived animals. In rhesus monkeys, a single injection of CT (30 U/kg) greatly suppressed feeding for three days and drinking for one day (both $p<0.01$). A retrospective study of humans demonstrated a 2% reduction in body weight ($p=0.03$) during the 12-36-hr following a single S.C. injection of CT (2 U/kg).

To test the hypothesis that these effects were due to a direct action on the brain, CT was administered intraventricularly to rats. On a dosage basis, these injections were approximately 75-fold more effective than S.C. injections in reducing feeding; amounts as small as 43 ng (0.2 U) significantly suppressed 24-hr food intake ($p<0.02$). Other experiments indicated that CT (12.5 U/kg, S.C.) effectively suppressed feeding during a 30-minute test only

when administered several hours prior to the test; injections one hour before testing were ineffective.

Thus, CT reduces feeding, apparently through a direct action on the brain. This action does not seem to be manifest immediately, but only several hours after its administration. It is, therefore, suggested that post-prandial secretion of CT acts on the brain to inhibit feeding during subsequent meals. Thus, when a meal is not eaten for any reason, the absence of normally concomitant CT secretion may serve to enhance feeding during subsequent meals.

XX. Tardive Dyskinesia

Tardive Dyskinesia is considered a major limitation to treatment with neuroleptics. There are, however, controversies regarding its incidence, etiopathology, course, prevention and treatment.

Twenty-one patients over the age of 50, who were admitted to New York (Westchester division) during a one-year period, and who had tardive dyskinesia were followed for a mean of 13 months. Neuroleptics and antidepressants were discontinued for at least three months. Dyskinetic symptoms abated in 12 patients, and persisted in 9. Discriminant function analysis showed that the persistent and reversible dyskinesia groups could be clearly separated and that the one best discriminator was the number of drug-free intervals. The persistent dyskinesia group had significantly longer neuroleptic treatment and greater number of drug interruptions than the reversible dyskinesia group. Our findings raise doubts about the commonly recommended practice of frequently interrupting long-term drug treatment, at least in patients who are otherwise predisposed to develop tardive dyskinesia.

We are presently conducting a series of animal experiments to test the effect of drug interruptions on a model of tardive dyskinesia. Initial experiments show that relatively short drug free periods do not reduce neuroleptic induced supersensivitiy of dopamine receptors, which is the presumed mechanism for production of tardive dyskinesia.

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TITLE OF PROJECT (80 characters or less)

Longitudinal Studies of Schizophrenic Behavior

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Thomas D. Reynolds — Chief, Section on Psychophysiology of Schizophrenia — SMRC

OTHER: None

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Clinical Psychopharmacology

SECTION
Section on Psychophysiology of Schizophrenia

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032

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XX (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Ongoing collection of daily behavior of 11 schizophrenics and two organic patients from WAW-4. Purpose is to explore evidence for homeostatic regulation in such data and to characterize such regulation mathematically and seek in basis of differentiating (1) clinical groups and (2) changes occurring with treatment changes in individual patients. Controlling hypothesis is that behavioral steady states are more difficult for schizophrenics to achieve and our basis of his progressive withdrawal. Data suggests hebephrenics exhibit most marked behavioral cycles. Focus will be on comparing effects on varying psychotropic drug dosage on time-series parameters (phenothiazines and lithium). Review of past data confirms spectrum peaks all hebephrenic records, none for paranoid and catatonic patients. Phase plane analysis yields unitary theoretical model for all data (modified three-variable volterra scheme).

PHS-6040
(Rev. 10-76)

Project Description:

Objectives:

To obtain a quantitative description of behavior which permits empirical construction of predictive models. It is hoped that such models can be used as the units of an input-output theory of behavior, with particular reference to the problem of behavioral stabilisty.

Methods Employed:

Behavioral notes are made 30 times daily on each of 14 chronic patients (12 schizophrenics, 2 organics for comparison purposes) on Ward 4 of the William A. White Building of Saint Elizabeths Hospital. The unit datum is a percentage of daily (or weekly) check-times positive for a given behavior (e.g., pacing, finger movement, talking, etc.). The resulting time-series is examined in a variety of ways to evidence for non-random components. The models developed from the data are used to predict into new data, and progressively refined. When a model has been shown to be consistently superior to others, it is regarded as a better or more valid representation of the process being studied. Both discrete time series approaches and stimulation techniques utilizing differential equations are being applied to the data.

Major Findings:

Work previously reported involved a variety of successful predictors for the time-series studied, but no uniform computational approach has been successful for all of the time-series. It becomes desirable, therefore, to develop an algorithm which would compete successfully with all previous individual predictors, so that each time-series could be meaningfully compared to another for the purpose of stronger statements about comparisons across patient type.

At present, polynomial expressions with Gaussion weights have shown to be superior to all other types of expression. However, they are markedly superior to linear modeling only in cases where clear periodical signals are present. Non-stationarity has emerged as a critical problem in this property. Evidence to date suggests that non-stationarity more strongly characterizes the organic group (three patients) than the schizophrenic group.

Five to six-day behavioral cycles have been identified in three of the four hebephrenic patients on Ward 4 and in none of the non-hebephrenic patients. Power spectra are the most efficient techniques for detecting steady cycles.

Preliminary data suggest that lithium may flatten the behavioral records of two patients with strong oscillations, corresponding to quantitative clinical improvement. This possibility will be systematically explored, since central to the entire study is the hypothesis of a hemostatic deficit in schizophrenia.

Significance to Bio-Medical Research and the Program of the Institute:

While the basic purpose of this study continues to be the ultimate development of input-output modeling of human behavior in specific environments, with particular reference to a study of disturbances of regulation in the major psychoses, it would appear that the overall methodology may be applicable to the study of response to psychotropic drugs, and perhaps yield some clues as to their mode of action in functional as opposed to biochemical terms. Overall strategy controlled by the hypothesis of a homeostatic deficit in schizophrenia. Hebephrenia seens biologically separable on the basis of current data, and supports concept of central homeostatic failure in these patients.

Proposed Course:

Continuation of (1) data collection; (2) theoretical modeling; and (3) evaluation of drug responses.

Publications:

Reynolds, T. D.: Quantitative studies of schizophrenia. Behav.Proc. 1: 347-372, 1976.

Reynolds, T. D.: Mathematical analysis of a behavioral rhythm in a schizophrenic man. Waking and Sleeping 1: 307-312, 1977.

Reynolds, T. D., London, W., and York, J.: Behavioral rhythms in schizophrenia. J.Nerv.Ment.Dis. (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 01337-07 SMRC

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Studies on Drugs of Abuse

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. J. Wyatt	Chief, Lab. of Clin. Psychopharmacology	SMRC
	R. C. Stillman	Research Psychiatrist	SMRC
	D. M. Stoff	Staff Fellow	SMRC
	E. V. DeRenzo	Psychologist	SMRC
OTHER:	O. Wolkowitz	Psychologist	SMRC
	R. Hawks	Chemist	NIDA
	P. Renault	Psychiatrist	NIDA
	D. Murphy	Psychiatrist	LCS
	H. Weingartner	Psychiatrist	APB

COOPERATING UNITS (if any)
Division of Research, National Institute on Drug Abuse; Laboratory of Clinical Science, National Institute of Mental Health; Adult Psychiatry Branch, National Institute of Mental Health

LAB/BRANCH
Laboratory of Clinical Psychopharmacology

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.85	2.5	1.35

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The long range purpose of this project continues to be determination of the mechanism of actions of drugs of abuse and the reasons why these drugs are used. We are interested in anything drug effects can help us understand about normal mental function or mental illness. Last year, we demonstrated marijuana's selective and differential effects on man's two cerebral hemispheres. This year, we have extended these studies to alcohol and to a greater variety of tests.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives:

To learn more about the mechanism of action and the reason why these drugs are abused.

Methods Employed:

Subjects were chronic marijuana users, usually adminstered natural marijuana cigarettes or a placebo cigarette prior to the biochemical or psychological testing. Under conditions of alcohol testing, subjects were given 1 ml 95% EtOH or a placebo prior to testing.

Major Findings:

A. Marijuana depressed the left more than the right hemisphere in a match-to-sample test. In a Posner nominally-same/physically-same paradigm, marijuana was also accompanied by a shift toward right hemispheric function, but alcohol seems to retard such a shift. In addition, a gradually progressing shift toward right hemisphere advantage in information processing occurred over successive days of testing.

In an oddity-detection task, a clear lateralization of stimuli was found, but marijuana did not change this at all. Instead, marijuana increased the latency of "different" judgments, while not at all affecting "same" judgments. Both hemispheres seemed to show this marijuana effect equally. Alcohol (1 ml/kg) did not increase latency, but increased error rates, especially "different" judgments. The two drugs therefore showed specific effects on information processing, different from one another.

Further studies will be undertaken to localize the effects of these drugs.

B. MAO Activity and Chronic Marijuana Use

Last year we showed that platelet MAO activity is significantly lower in marijuana users than non-users, and lower in heavy users than in light users. We collected samples of blood from Costa Rican citizens who were known to be heavy marijuana smokers (up to 75 "joints" a day). They are being analyzed in several laboratories for, since this population level of heavy use is considerably different than found in this country, the specimens, when analyzed, should help interpret our earlier findings.

C. Cooperative Studies of THC Analytic Methodologies

We administered a structured interview and questionnaire to 100 consecutive subjects in our experiments to study their profiles of drug preference

and use. We have uncovered several highly significant relationships which may be quite important in understanding patterns of drug abuse.

We have found that the specific effects which a subject seeks from marijuana are very highly correlated with his choice of other drugs. Subjects who look to marijuana for increased insights, ideas, and a deeper involvement in music, people, etc., almost always prefer amphetamines to tranquilizers and barbiturates. Subjects who, on the other hand, use marijuana chiefly to relax strongly prefer the sedative/hypnotic drugs to stimulants. An interesting and important exception to this rule is cocaine, which is very much enjoyed, despite its stimulant qualities, by most of the sedative-seeking group. Another drug with considerable popularity in both groups is alcohol. We suspect that part of the appeal of marijuana, alcohol and cocaine is the ability of these drugs to create different effects depending on the user and the setting. Thus they are polyvalent drugs which can be used by different people in the same social setting to produce individually sought and varied effects.

D. Cooperative Studies of THC Analytic Methodologies

In collaboration with several NIDA contractors we have conducted cooperative validation studies which show RIA (radioimmunassay) techniques to be reasonably close in accuracy to much costlier GC/MS analyses of delta-9-THC in human blood.

E. Animal Studies

Phencyclidine ("angel dust") and amphetamine ("speed") are two of the most widely abused drugs. Both have CNS stimulant properties which may be mediated through dopamine. An animal model for the long-lasting residual effect of these drugs does not exist. We are currently testing the long-term effects of phencyclidine and amphetamine on the following paradigms: chronic activity, stereotypy, turning behavior, and avoidance.

Publications:

Stillman, R. C., Wolkowitz, W., Weingartner, H., Waldman, I., DeRenzo, E. V. and Wyatt, R. J.: Differential effects of right and left hemisphere functions in man. Life Sci. 21: 1793-1800, 1977.

Petersen, R. and Stillman, R. C.: Phencyclidine (PCP) Abuse. Government Printing Office, Washington, D.C., (in press).

Stillman, R. C. and Petersen, R.: Phencyclidine (PCP) Abuse: A Summary. National Institute on Drug Abuse, Washington, D.C., (in press).

Stillman, R. C., Wyatt, R. J., Murphy, D. L. and Rauscher, F. P.: Low Platelet monoamine oxidase activity and chronic marijuana use. Life Sci. (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 01339-03 SMRC

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Eye Tracking in Schizophrenic Patients

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Thomas D. Reynolds Chief, Section on Psychophysiology of Schizophrenia SMRC

OTHER: None

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Clinical Psychopharmacology

SECTION
Section on Psychophysiology of Schizophrenia

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.2	0.1	0.1

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Eye tracking of schizophrenic and normal controls is being explored. Pilot study suggests simple attentional deficits, correctable by making stimulus situation more interesting.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives:

To attempt replication of reported finding of defective eye-tracking in schizophrenia.

Methods Employed:

Electrooculogram is recorded while subject follows a pendulum movement. Various stimuli are affixed to pendulum bob.

Major Findings:

Over 40 patients have been studied. Eye-tracking in schizophrenia most markedly improved by affixing picture of a face on the bob. Similar findings have been reported, but our data suggest schizophrenia tracking more closely approximate normal tracking than hitherto reported.

Significance to Bio-Medical Research and the Program of the Institute:

Utility of eye-tracking as a genetic marker rendered doubtful. However, as a sensitive technique for recording attentional deficit and the variables which influence the latter, the tracking task may be ideal. It will be pursued with more practical goals in mind (i.e., a technique for monitoring treatment response).

Proposed Course:

We expect to continue to use apparatus as part of functional test battery used to monitor treatment response.

Publications:

None.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 01257-06 SMRN

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)
Studies on the generation of epileptic form activity in the hippocampal slice.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: A. P. Oliver SMRN NIMH

Other: J. S. Carman M.D. SMRC NIMH

COOPERATING UNITS (if any)
Laboratory of Clinical Psychopharmacology

LAB/BRANCH
Laboratory of Neuropharmacology

SECTION
Office of the Chief, Laboratory of Neuropharmacology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, St. Elizabeths Hospital, Washington, D.C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

An in vitro technique, the hippocampal slice, has been used to study ionic mechanisms in the generation of epileptiform activity. It has been found that a complex interaction between potassion and calcium ions is major factor in determining the generation and duration of seizures in this model system.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives:
The slice preparation is used to characterize the interaction between known concentrations of various substances and the physiological properties of organized brain tissue.

Methods Employed:
Hippocampi from guinea pigs are dissected out of the brain and sliced so as to maintain their anatomical structure. The slices are placed in a chamber designed for this purpose. The slice retains most of its normal physiology for several hours. The cells are spontaneously active and can be stimulated for experimental purposes. Quantities of drugs or other substances can be altered in the bathing medium.

Major Findings:
1. The slice can be made to generate epileptiform activity by penicillin application. 2. This activity can be used to screen the effects of anticonvulsant drugs in a quantitative manner. 3. Appropriate levels of potassium and calcium ions are necessary to produce and maintain seizures. 4. An increase in K ion is quantitatively related to the severity of seizures as tested in this model. 5. Increases or decreases of calcium ion followed by a return to control levels have a powerful and longlasting effect which increases the tissue excitability. 6. Stable increases or decreases in calcium ions result in stable alterations of excitability. 7. The tissue is sensitive to alterations of calcium level as small as 10%. 8. Lithium ion causes an increase in excitability in the tissue. 9. Lithium ion does not block or facilitate effects produced by altering calcium ions. 10. Magnesium ion reduces seizure activity when its concentration is increased. 11. All the ions were tested at concentrations compatible with those found in vivo.

Proposed Course:
The Hippocampal Slice: A system for studying the pharmacology of seizures and for screening anticonvulsant drugs was accepted for publication in the journal Epilepsia 18 (4) 1977. A paper describing the quantitative study with potassion ion has been submitted to Experimental Neurology. Two papers describing the calcium and lithium studies are in preparation. Further studies on ionic mechanisms are being pursued.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 01300-04 SMRN

PERIOD COVERED
October 1, 1977 to September 30, 1978.

TITLE OF PROJECT (80 characters or less)
A Combined Electron Microscopic, Histochemical and Electrophysiological Study of Synaptic Innervation.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Forrest F. Weight M.D.,Chief, Section on Synaptic Pharmacology SMRN NIMH

Other: H. Weitsen Ph.D.

COOPERATING UNITS (if any)

Department of Anatomy, LSU Medical Center, New Orleans, Louisiana.

LAB/BRANCH
Laboratory of Neuropharmacology

SECTION
Synaptic Pharmacology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, St. Elizabeths Hospital, Washington, D.C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A combination of electron microscopic, histochemical and electrophysiological methods were utilized to investigate the synaptic organization of the ninth and tenth paravertebral sympathetic ganglia of the bullfrog. The neurons were unipolar and were innervated by a single preganglionic axon. The cholinergic preganglionic fiber stained for acetylcholinesterase and was observed in both the light and electron microscope to spiral around the axon hillock of the ganglion cell before arborizing and making synaptic contact with the neuron. Most synapses were located on the soma near the axon hillock region, with electron microscopic features typical for cholinergic junctions. The axosomatic location of the synapses was manifested physiologically by a decrease in membrane resistance (increased conductance) at the peak of the fast EPSP and by a demonstrable reversal potential for the fast EPSP.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives:

Synaptic transmission has been investigated in considerable detail in sympathetic ganglia. These ganglia are of particular interest because several types of synaptic potentials can be recorded, including a fast EPSP, a slow EPSP and a slow IPSP. The latter two are of particular interest because of their different mechanisms of generation. Much of our current understanding of the generation mechanisms of these synaptic potentials is derived from electrophysiological investigations on the ninth and tenth sympathetic ganglia of the bullfrog. There is, however, relatively little detailed morphological information on the synaptic organization of these ganglia. In view of this, we used a combination of histochemical and electron microscopic techniques to obtain additional information on the organization and fine structure of the innervation of these neurons. In addition, intracellular recording was used to study the physiological correlates of the synaptic organization.

Methods Employed:

In this study a combination of histochemical, electron microscopic and electrophysiological methods were used to study the ninth and tenth paravertebral sympathetic ganglia of the bullfrog.

For light microscopic histochemistry, the ganglia were excised, placed in a cryostat chuck and frozen quickly in isopentane cooled by dry ice. The tissues were maintained at a temperature of -22°C and sectioned at a thickness of 10-14 µM, placed on glass slides and air dried. The tissues were then fixed in 2-4% buffered formaldehyde and stained for acetylcholinesterase by the method of El-Bodawi and Schenk.

For electron microscopic investigation, the ganglia were fixed in situ by perfusion with buffered 3% gluteraldehyde and 1% formaldehyde. The tissues were post-fixed with 1% osmium tetroxide and subsequently prepared with standard procedures for electron microscopy. In experiments to determine the ultrastructural localization of AChE, an enzyme incubation step was added before treatment with osmium. Examination and photography of the thin sections were performed on Phillips 200, 300, and Zeiss 9S electron microscopes.

Intracellular recording in neurons of these sympathetic ganglia utilized standard electrophysiological techniques; the microelectrodes were filled with 3 M KCl. Preganglionic nerves were stimulated by means of suction electrodes on both the sym-

pathetic chain and the eighth spinal nerve. A similar electrode on the tenth nerve was used for antidromic stimulation. The ganglia were continuously superfused with oxygenated Ringer's solution.

Major Findings:

Light microscopic observation revealed the ganglion cells to be unipolar neurons. Acetylcholinesterase (AChE) activity was particularly localized to the region of the axon hillock and the adjacent neuronal soma. In some sections, the AChE localization was observed in a spiral around the axon hillock region of the neuron. The localization of AChE was also studied by electron microscopy. AChE was localized on the axolemma of the preganglionic axon and in the cleft around the synaptic terminals.

The find structure of the innervation showed that the preganglionic axon spirals around the axon hillock before making synaptic contact with the ganglion cell. The majority of synapses were found on the region of the soma near the axon hillock and on the axon hillock. The synaptic terminal had the electron microscopic appearance typical for cholinergic junctions.

The physiological correlates of the axosomatic location of synapses were studied by intracellular recording. In most neurons, the EPSP is all-or-none, indicating innervation by one preganglionic fiber. A test of membrane resistance shows that membrane resistance is markedly reduced at the peak of the EPSP. Additionally, electrical polarization of membrane reverses the EPSP at a membrane potential near -16 mV.

Significance to Bio-medical Research and the Program of the Institute:

The combination of anatomical and physiological techniques used in this investigation has provided information on the synaptic innervation of the sympathetic ganglion cells of the bullfrog. The all-or-none characteristics of the EPSP indicates that the neurons are innervated by one preganglionic fiber. The acetylcholinesterase histochemistry and the electron microscopy show that the preganglionic fiber spirals around the axon hillock, arborizing and making synaptic contacts on the soma of the neurons, particularly near the axon hillock. Since, in most neurons, the action potential originates in the region of the axon hillock, this anatomical location of synapses appears particularly well suited for the synaptic activation of action potentials.

The location of synapses on the soma and axon hillock region

of these sympathetic neurons makes these neurons particularly well suited for investigating the ionic basis of synaptic potential generation. Frequently, the conductance changes underlying the generation of synaptic potentials cannot be adequately investigated in vertebrate neurons because the synaptic junctions are remote from the soma. As a result, it may be difficult or impossible to detect a conductance change associated with the EPSP. Furthermore, it also may not be possible to determine a clear cut equilibrium or reversal potential for the EPSP. As shown in this investigation, the neurons in sympathetic ganglia of bullfrogs are monopolar and do not have dentrites. Additionally, the synapses are predominantly located on the soma. One would therefore expect to be able to demonstrate the conductance changes generating the synaptic potentials in these neurons. The physiological investigations in this project confirm that this is indeed the case. The increase conductance that generates the fast EPSP is demonstrated by the decrease in membrane resistance at the peak of the fast EPSP. Furthermore, the somatic location of synapses is also manifested by the ability to reverse the fast EPSP by electrical polarization of the membrane. On the basis of the combined anatomical and physiological data in this study, it is concluded that the sympathetic ganglion of the bullfrog is an excellent preparation for investigating transmission at neuronal synapses.

Proposed Course:

This project will be terminated in NIMH due to the reassignment of the P.I. to NIAAA.

Publications:

Weitsen, H.A. and Weight, F.F.: Synaptic innervation of sympathetic ganglion cells in the bullfrog. Brain Res. 128: 197-211, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 01303-04 SMRN

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Studies on Physiological Mechanisms Modulating the Release of Synaptic Transmitter.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Forrest F. Weight M.D., Chief, Section on Synaptic Pharmacology SMRN NIMH

Other: S. D. Erulkar Ph.D.

COOPERATING UNITS (if any)
Department of Pharmacology, University of Pennsylvania Medical School, and Marine Biological Laboratory, Woods Hole, Massachusetts.

LAB/BRANCH
Laboratory of Neuropharmacology

SECTION
Synaptic Pharmacology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, St. Elizabeths Hospital, Washington, D.C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The effect of repetitive action potentials in the postsynaptic axon on the release of synaptic transmitter from the presynaptic terminal was investigated at the squid giant synapse. Repetitive antidromic stimulation of the postsynaptic axon resulted in a reduction in EPSP amplitude. The reduction in transmitter release was accompanied by a decrease in the presynaptic spike after-hyperpolarization (AH). Increasing the concentration of extracellular potassium ions also reduced the EPSP and decreased the amplitude of the presynaptic spike AH. The reduction in transmitter release resulting from repetitive postsynaptic impulses is attributed to the accumulation of extracellular potassium ions. The accumulation of extracellular potassion ions may thus modulate synaptic transmission and function as an integrative mechanism in the nervous system.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives:

The modulation of synaptic transmitter release may play an important role in the processing of information in the nervous system. Mechanisms which have been reported to modulate synaptic transmission include repetitive activity in the presynaptic terminals and the presynaptic action of other synapses. There has been, however, no evidence for a direct postsynaptic role in the modulation of transmitter release. This fact, together with evidence that increasing the extracellular potassium (K) concentration depressed transmitter release, prompted us to investigate whether the accumulation of extracellular K resulting from a train of postsynaptic impulses might modulate transmitter release.

Methods Employed:

The stellate ganglion was dissected from the squid, Loligo pealii, and placed in a small Lucite chamber perfused by oxygenated artificial seawater. The bath temperature was lowered until the EPSP no longer initiated spike generation. Experiments were then performed at a constant temperature ($\pm$ 0.1^{o}C) between 6 and 12 C. Standard electrophysiological techniques were used to record from microelectrodes placed intracellularly in the midregion of the presynaptic terminal and in the postsynaptic axon near the synapse.

Major Findings:

Repetitive antidromic action potentials in the postsynaptic axon produced a depression in the release of synaptic transmitter from the potentials, EPSP amplitude was reduced. The average percentage reduction in EPSP amplitude was 27.6 $\pm$ 7.9 (s.d.m.; n=9) for postsynaptic stimulation at a frequency of 25 Hz for 20 seconds.

The repetitive impulses in the postsynaptic axon also reduced the amplitude of the presynaptic spike AH. In five experiments, when the ratio of change in presynaptic spike AH to change in postsynaptic spike AH was compared for different stimulus durations, the ratio for a given synapse varied by less than 0.1. These data indicate that the K that accumulates in extracellular spaces around the postsynaptic axon also affects the presynaptic terminal.

Increasing extracellular K also produced a reduction in the amplitude of both the EPSP and the presynaptic spike AH. On the average, increasing extracellular K resulted in a 20% change in EPSP amplitude per mV change in presynaptic spike AH. With

repetitive antidromic stimulation of the postsynaptic axon, the average ratio of percentage change of EPSP amplitude to change of AH was 27.6 + 4.3 (s.e.m.; n=31). This suggests that the reduction in EPSP amplitude resulting from repetitive postsynaptic action potentials is due to the accumulation of extracellular K ions around the presynaptic terminal.

Significance to Bio-medical Research and the Program of the Institute:

The modulation of transmitter release by repetitive postsynaptic action potentials can function as a type of negative feedback to inhibit impulse transmission through a synapse. Previous investigations have shown negative feedback through synaptic inhibition of an output neuron, but not a direct feedback on synaptic input. This investigation provides evidence for a direct inhibitory feedback from the postsynaptic structure that modulates transmitter release from the presynaptic terminal. In this way, the accumulation of extracellular K resulting from physiological activity may modulate synaptic transmission and function as an integrative mechanism in the nervous system.

Proposed Course:

This project will be terminated in NIMH due to the reassignment of the P.I. to NIAAA.

Publications:

Weight, F. F. and Erulkar, S. D., Modulation of Synaptic Transmitter Release by Repetitive Postsynaptic Action Potentials. Science 193: 1023-1025, 1976.

Weight, F. F., The Modulation of Synaptic Transmission and Information Processing in the Nervous System. In: IEEE, Conference on Cybernetics and Society, 1976, pp 285-299.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 01304-02 SMRN

PERIOD COVERED
October 1, 1977 to September 30, 1978.

TITLE OF PROJECT (80 characters or less)
The Role of Potassium in the Control of Synaptic Transmitter Release.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI.: Forrest F. Weight M.D., Chief, Section on Synaptic Pharmacology SMRN NIMH

Other: S. D. Erulkar Ph.D.

COOPERATING UNITS (if any)
Department of Pharmacology, University of Pennsylvania Medical School, and Marine Biological Laboratory, Woods Hole, Mass.

LAB/BRANCH
Laboratory of Neuropharmacology

SECTION
Synaptic Pharmacology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, St. Elizabeths Hospital, Washington, D.C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The effects of changes in extracellular potassium (K) concentration on synaptic transmission were studied at the squid giant synapse with intracellular recording from the presynaptic terminal and the postsynaptic axon. The amplitudes of both the presynaptic spike and the EPSP varied inversely with extracellular K. On the average, a 10 mV change in spike height was accompanied by a 3.1 mV change in EPSP amplitude. The amplitude of the presynaptic spike after-hyperpolarization varied inversely with extracellular K. On the average, increasing extracellular K resulted in a 20% change in EPSP amplitude per mV change in presynaptic spike after-hyperpolarization. The effect of extracellular K on EPSP amplitude appears to be mediated by changes in the amplitude of the presynaptic spike.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives:

Potassium (K) ions have been shown in previous investigations to accumulate extracellularly with repetitive nervous activity. There is little information, however, on the functional significance of such increases in extracellular K. In this investigation, we studied the effect of changing extracellular K concentration on synaptic transmission.

Methods Employed:

The experiments were conducted on the giant synapse of the squid, Loligo pealii, at the Marine Biological Laboratory in Woods Hole. The stellate ganglion was dissected, placed in a small lucite chamber and constantly perfused with oxygenated sea water. Artificial sea water with a K concentration of 9 mM was changed by addition to or substraction from the artificial sea water, without adjustment of the other ions. Postsynaptic spike initiation was prevented by cooling the preparation sufficiently to reduce the EPSP below threshold.

The recording microelectrodes were filled with 3 M KCl. One microelectrode was placed in the postsynaptic giant axon close to the synapse, the other electrode in the mid-region of the presynaptic terminal. Standard electrophysiological techniques were used for recording. Traces on two oscilloscopes were photographed on separate cameras. Measurements were made from enlarged photographic images on an analog to digital converter (Oscar, Model F). Calculations were performed on a PDP-12 computer. Least squares analysis of exponential functions were performed on a PDP 10 computer.

Major Findings:

An increase in extracellular K from 9 mM in the artificial sea water (ASW) to steady levels of 10.5 mM and 12 mM, progressively decreased the amplitude of the EPSP. Conversely, decreasing extracellular K to 6 and 3 mM progressively increased the amplitude of the EPSP. Within the range of extracellular K tested (3-12 mM), the relationship between extracellular K and the percentage change in EPSP amplitude was linear; a 24.5% change in EPSP amplitude occuring per mM change in extracellular K.

Since K conductance is high during the spike after-hyperpolarization (AH), the membrane behaves as a K electrode and the amplitude of the AH can be taken as an index of

extracellular K concentration. The ratio of percentage change in EPSP to change in AH can therefore be used to assess whether a change of transmitter release results from the accumulation of extracellular K. This relationship for increasing extracellular K gives a 20% change in EPSP amplitude per mV change in presynaptic spike AH.

One mechanism that could account for the effect of extracellular K on EPSP amplitude is that it may be mediated by changes in the amplitude of the presynaptic spike. Such presynaptic changes were observed. There was a 3.1 mV change in EPSP amplitude per 10 mM change in spike height. The results of the present experiments indicate that the change in EPSP amplitude produced by changing extracellular K can be accounted for by the change in presynaptic spike height. The relationship between presynaptic spike height and EPSP amplitude agrees essentially with data from experiments by others on the same preparation using different techniques to alter the amplitude of the presynaptic spike.

Significance to Bio-medical Research and the Research of the Institute:

Although repetitive action potentials are known to result in the accumulation of extracellular K in nervous tissues, there is little information on the physiological effects and functional significance of increased extracellular potassium. This investigation shows that the effects of changes in extracellular K concentration on synaptic transmission. Increased extracellular K can depress synaptic transmitter release from presynaptic terminals. This could be important functionally when nervous activity results in an accumulation of extracellular K.

Proposed Course:

This project will be terminated in NIMH due to reassignment of the PI to NIAAA.

Publications:

Erulkar, S.D. and Weight, F.F. Extracellular potassium and Transmitter Release at the Giant Synapse of Squid. J. Physiol. 266: 209-218, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 01305-02 SMRN

PERIOD COVERED
October 1, 1977 to September 30, 1978.

TITLE OF PROJECT (80 characters or less)

The Role of Temperature in Synaptic Transmitter Release.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Forrest F. Weight M.D., Chief, Section on Synaptic Pharmacology SMRN NIMH

Other: S. D. Erulkar Ph.D.

COOPERATING UNITS (if any)
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SECTION
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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	0

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Lowering the temperature of the squid giant synapse prevents EPSP initiation of the postsynaptic action potential and permits investigation of the relationship between the EPSP and the presynaptic action potential. This study revealed that transmitter release is temperature sensitive; from an Arrhenius plot, an apparent activation energy of 74 kcalorie per degree, was calculated. A change in presynaptic spike height does not appear to be the major factor involved in the sensitivity of transmitter release to temperature. The effect of temperature on transmitter release is therefore likely to involve other factors such as temperature sensitivity of the release mechanism.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives:

There have been several previous investigations of the effects of temperature on synaptic transmitter release at different synaptic junctions. Although all reports agree that synaptic transmission is affected by temperature, detailed analysis of the factors involved has not been possible because the relationship between presynaptic events (such as spike amplitude) to postsynaptic response could not be investigated. It is possible to study this relationship at the squid giant synapse where intracellular recording can be obtained from both the presynaptic terminal and the postsynaptic axon. In this study we used to squid synapse investigated the effect of temperature on the presynaptic spike and the EPSP.

Methods Employed:

The stellate ganglion was dissected from the squid, Loligo pealii, placed in a small Lucite chamber and perfused continuously with oxygenated seawater. The temperature of the bath was controlled by a thermoelectric device and continuously monitored. Micropipette electrodes filled with 3 M KCl were inserted in the mid-portion of the presynaptic terminal and in the postsynaptic axon near the synapse. Conventional electrophysiological recording techniques were used.

Major Findings:

At room temperature (18-20°C), the EPSP in the squid axon almost always generates a postsynaptic action potential. In all 43 synapses investigated, when the temperature was lowered sufficiently, initiation of postsynaptic spike generation by the EPSP did not occur. The mean temperature at which the EPSP failed to activate a spike was 8.9 $\pm$ 1.5 (s.d.m.) and the range was from 5.6 to 12°C.

When temperature was decreased below the point at which the postsynaptic spike was activated, EPSP amplitude decreased progressively. This was accompanied by an increase in the width of the presynaptic spike due to a decrease in the rate of rise and fall of the action potential. Additionally, an increase in synaptic delay was also observed.

The relationship between temperature and the log of EPSP amplitude was linear with a high degree of temperature sensitivity. From an Arrhenius plot of the log of EPSP amplitude against the reciprocal of temperature (K), an apparent activation energy of 74 kcalorie per degree was calculated.

The greatest change in EPSP amplitude was in the temperature range between 9 and 10°C. In contrast to this, the largest change in presynaptic spike height was in the temperature range between 6 and 8°C. The comparison between change in presynaptic spike height and EPSP amplitude, as a function of temperature, does not agree with this relationship reported in previous studies using electrical polarization of the presynaptic terminal. This suggests that changes in the presynaptic spike height may not be a major factor in the effect of temperature on transmitter release.

Significance to Bio-medical Research and the Program of the Institute:

It is important to elucidate the mechanisms by which the release of synaptic transmitter from nerve terminals is controlled, in order to understand the cellular basis of nervous system function. In previous investigations on the effects of temperature on transmitter release, it has not been possible to study presynaptic mechanisms. Using the squid giant synapse in order to study presynaptic release mechanisms, we found that a change in presynaptic spike height does not appear to be the major factor involved in the sensitivity of transmitter release to temperature. The effect of temperature on transmitter release is therefore likely to involve other factors such as temperature sensitivity of the release mechanism.

Proposed Course:

This project will be terminated in NIMH due to the re-assignment of the P.I. to NIAAA.

Publications:

Weight, F.F. and Erulkar, S.D.: Synaptic transmission and effects of temperature at the squid giant synapse. Nature 261: 720-722, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 01306-02 SMRN

PERIOD COVERED
Oct. 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)
Fluorescence Histochemistry and Electron Microscopy of Small Intensely Fluorescent (SIF) Cells

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Fluorescence histochemical and electron microscopic techniques were used to investigate small intensely fluorescent (SIF) cells in the ninth and tenth paravertebral sympathetic ganglia of bullfrogs. Fluorescence histochemistry revealed that clusters of SIF cells are sparsely distributed in the ninth and tenth ganglia; the clusters were usually located in the vicinity of blood vessels. Fluorescent processes were not observed emanating from SIF cells. The clusters stained positively for the chromaffin reaction indicating that SIF cells are chromaffin cells. Ultrastructurely, the SIF-chromaffin cells in the sympathetic ganglia appeared virtually identical to the chromaffin cells in the adrenal gland; this includes two cell types that appear morphologically the same as the epinephrine and norepinephrine containing chromaffin cells in the adrenal gland. Efferent synapses from the SIF-chromaffin cells in sympathetic ganglia to sympathetic neurons were not observed. The SIF-chromaffin cells in bullfrog sympathetic ganglia did not have the morphological characteristics of interneurons; it is suggested that they may function as extra-adrenal chromaffin tissue.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives:

Small intensely fluorescent (SIF) cells have been reported in the sympathetic ganglia of several species. On the basis of electrophysiological studies on rabbit superior cervical ganglia, it has been proposed that SIF cells may be adrenergic interneurons in the pathway that mediates the slow IPSP. The bullfrog sympathetic ganglion is particularly well suited for investigating the morphologic basis of the slow IPSP because it is generated in a separate cell type by stimulation of a specific synaptic pathway. Furthermore, much of our current understanding of the mechanism of generation of the slow IPSP derives from investigations on bullfrog sympathetic ganglia. There is, however, relatively little morphological information on the synaptic organization of this preparation. We therefore initiated this investigation in an attempt to demonstrate morphologically the specific anatomical pathway mediating the generation of the slow IPSP.

Methods Employed:

Small intensely fluorescent (SIF) cells were identified using the Falck-Hillarp fluorescence method for the cellular localization of biogenic amines. Sympathetic ganglia were excised and frozen in isopentane cooled with dry ice. The ganglia were freeze-dried for 3-5 days and subsequently treated for 2-3 hours at 80°C with paraformaldehyde vapor. The ganglia were vacuum infiltrated with Maraglas which was then polymerized at 60°C overnight. Serial sections 1 micron in thickness, were cut, examined on a Zeiss fluorescence microscope, and individual SIF cells or SIF cell clusters were identified.

In order to further characterize the morphology of the SIF cells and to be able to recognize them in plastic embedded sections prepared for electron microscopy, the sections containing SIF cells identified in the fluorescence microscope were stained with toluidine blue; the sections were then reexamined using bright field microscopy and the morphological characteristics of the identified SIF cells, stained with toluidine blue, were studied.

Because of the sparse distribution of SIF cells in these ganglia, serial sections, 1 micron in thickness, of the plastic embedded tissue were stained with toluidine blue and light microscopically examined for SIF cells. When cells or clusters were identified, thin sections for electron microscopy were taken. In this manner, it was possible to locate SIF cells and to study their ultrastructural characteristics. The tissue was examined and photographed using Phillips 200,300, and Zeiss 9S

electron microscopes.

Major Findings:

As indicated by their name, small intensely fluorescent (SIF) cells are characterized by their very bright fluorescence. These cells were relatively infrequent in the ninth and tenth paravertebral sympathetic ganglia of the bullfrog. They usually occurred in small clusters containing 3-15 cells. The SIF cell clusters were randomly distributed throughout the ganglia and were usually located in the vicinity of blood vessels. Fluorescent processes were not observed emanating from any individual SIF cells or from SIF cell clusters. The morphology of these cells was characterized further by staining with toluidine blue. When stained in this manner and viewed with normal bright field microscopy, the cells were found to be small, 10-20 microns in diameter, rounded or ovoid, with peripherally located aggregates of heterochromatin, and granular cytoplasm.

Due to the morphological similarities of ganglionic SIF cells and adrenal chromaffin cells, the question arose whether SIF cells are indeed chromaffin cells. Chromaffin cells in the adrenal gland are characterized by a positive chromaffin reaction. We therefore employed this reaction on sympathetic ganglia to determine if SIF cells are chromaffin positive. We found that SIF cells in sympathetic ganglia stain positively with the chromaffin reaction, indicating that they are indeed chromaffin cells.

Electron microscopic observation of the SIF-chromaffin cells reveals that they contain an abundance of large electron-dense granules. On the basis of the appearance of the granules, two cell types can be distinguished; one with very electron-dense granules, and the other with granules of moderate density. Additionally, axon-like cytoplasmic processes were not observed emerging from any chromaffin cells. Furthermore, no processes of chromaffin cells were seen penetrating the connective tissue capsule surrounding the clusters of chromaffin cells. In addition, chromaffin cells in the adrenal gland were also examined and appeared virtually identical to the SIF-chromaffin cells found in the sympathetic ganglia.

Significance to Bio-medical Research and the Program of the Institute:

In the present investigation, we studied the distribution and morphology of SIF cells in the ninth and tenth paravertebral sympathetic ganglia of the bullfrog in relation to their functional significance. Morphologically and histochemically the SIF cells appeared identical to chromaffin cells in the

adrenal glands. These observations suggest that SIF cells represent extra-adrenal chromaffin tissue located in sympathetic ganglia. In the present study, we are unable to find morphological evidence that SIF cells in bullfrog sympathetic ganglia makesynaptic connections with the neurons. The SIF-chromaffin cells did not have the morphological characteristics of interneurons and thus would not function as adrenergic interneurons in synaptic pathways. This is in accord with physiological data the fast and slow synaptic potentials are due to cholinergic innervation of the neurons.

Proposed Course:

This project will be terminated in NIMH due to the reassignment of the P.I. to NIAAA.

Publications:

Weight, F.F. and Weitsen, H.A.: Identification of small intensely fluorescent (SIF) cells as chromaffin cells in bullfrog sympathetic ganglia. Brain Res. 128: 213-226, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 01307-02 SMRN

PERIOD COVERED
October 1, 1977 to September 30, 1978.

TITLE OF PROJECT (80 characters or less)
Electrical Excitability of Neuronal Membranes and the Actions of Calcium and Theophylline.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Forrest F. Weight	M.D., Chief, Section on Synaptic Pharmacology	SMRN NIMH
Other:	P. A. Smith	Ph.D.	SMRN NIMH
	N. A. Busis	M.D.	
	C. Swenberg	Ph.D.	

COOPERATING UNITS (if any)
None

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SECTION
Synaptic Pharmacology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, St. Elizabeths Hospital, Washington, D.C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.9	0.7	0.2

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The electrical excitability of neuronal membrane was studied using the sucrose gap technique to investigate the sympathetic ganglion of the bullfrog. Action potentials were elicited in three ways: by antidromic stimulation across the sucrose gap; and by orthodromic stimulation through two separate presynaptic pathways. The effects of calcium ions and theophylline were studied on the electrogenic processes involved in action potential generation. We found that the spike after-hyperpolarization in these neurons has a calcium-sensitive component. The calcium sensitive component was found to be an increase in potassium permeability. Furthermore, this calcium-sensitive potassium conductance is potentiated by theophylline, a drug known to affect cellular calcium metabolism.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives:

In many neurons, calcium as well as sodium enters during the action potential. In previous studies, intracellular injection of calcium has been shown in several types of neurons to increase membrane permeability to potassium, and voltage clamp experiments have demonstrated that inward calcium currents can activate outward potassium currents. The action potential in many nerve cells is followed by a lower after-hyperpolarization, which results from increases of one or more potassium conductances. Therefore, calcium influx during an action potential might activate part or all of the subsequent after-hyperpolarization. The objective in this study was to investigate whether sympathetic neurons have a calcium-sensitive component involved in the electrogenesis of the spike after-hyperpolarization. In addition, the action of theophylline, a drug known to affect cellular calcium metabolism, was studied on the spike after-hyperpolarization.

Methods Employed:

The experiments were conducted on the ninth and tenth paravertebral sympathetic ganglia of the bullfrog by means of sucrose gap or intracellular recording. The ganglia were continuously superfused with an oxygenated Ringer's solution. In experiments studying the effect of calcium removal on neuronal electrogenic processes, calcium-free Ringer's solution was prepared by omission of calcium chloride. The action of theophylline on electrogenic processes was studied by superfusion in the Ringer's solution using a concentration of 5 mM.

Major Findings:

The action potential after-hyperpolarization in sympathetic neurons was considerably and reversably potentiated by theophylline. The after-hyperpolarization was increased whether the action potentials were generated by othodromic B or C fiber stimulation or by antidromic stimulation of the neurons. The predominant effect of theophylline was an enhancement of the after-hyperpolarization duration. It increased the half-time for decay of the antidromically elicited after-hyperpolarization up to 800%. The duration of the potentiated after-hyperpolarization was inversely dependent on stimulus frequency, with maximal duration observed only when stimuli were administered more than one minute apart. Theophylline enhanced the after-hyperpolarization amplitude to a lesser extent, only increasing the peak amplitude of the antidromically elicited after-hyperpolarization up to 150%. Theophylline did not significantly affect the amplitude of the action potential elicited by otho-

dromically elicited action potential.

The action potential after-hyperpolarization in these sympathetic neurons has been shown previously to be generated by an increase in potassium conductance, and thus is decreased by hyperpolarizing current across the membrane. In this investigation, it was found that hyperpolarization of the resting membrane potential progressively reduced and abolished the theophylline potentiated after-hyperpolarization, as it does the normal after-hyperpolarization. These data indicate that the potassium conductance during the after-hyperpolarization is prolonged by theophylline. In addition, these data distinguish the theophylline potentiated after-hyperpolarization from the slow IPSP, which is potentiated by moderate hyperpolarizing current.

The potentiation of the spike after-hyperpolarization by theophylline was abolished by the removal of extracellular calcium ions. This was shown in two ways. First, the ganglia were superfused with a Ringer's solution containing normal calcium and theophylline until maximal after-hyperpolarization potentiation occured. Then they were superfused with Ringer containing theophylline but no calcium. In the absence of extracellular calcium, the after-hyperpolarization was markedly diminished in duration and amplitude, becoming even smaller than the pre-theophylline control. The potentiated after-hyperpolarization returned when calcium was restored. Second, ganglia were superfused with calcium-free Ringer for more than 4 hours. At that time, the addition of theophylline did not significantly alter the antidromically elicited after-hyperpolarization. The calcium antagonists, magnesium and cobalt blocked the effect of theophylline on the spike after-hyperpolarization. Strontium and to some extent barium acted as calcium agonists. Theophylline was thus able to potentiate the spike after-hyperpolarization in a calcium-free strontium solution. Dantrolene (5mM) a drug which prevents the release of intracellular calcium did not block the effect of theophylline on the spike after-hyperpolarization. These data suggest that theophylline may be modifying the influx of calcium which occurs in the action potential. This possibility is currently being examined by attempting to directly observe the influx of calcium in an action potential. Using intracellular recording, an action potential can be elicited in an isotonic solution of calcium chloride and the effect of theophylline on this calcium spike can be studied.

Significance to Bio-medical Research and the Program of the Institute:

Our data show that the repolarization phase of the action potential after-hyperpolarization in these neurons depends on

extracellular calcium ions. It has been demonstrated previously that calcium as well as sodium ions can enter these neurons during the action potential. This suggest that calcium influx during the action potential activates a potassium conductance that prolongs the duration of the after-hyperpolarization. Alternatively, calcium influx may control the rate of inactivation of the after-hyperpolarization potassium conductance. In the present experiments, we also found that theophylline greatly potentiates the calcium-sensitive potassium conductance. Theophylline may increase stimulus-induced entry of calcium into these neurons, as it does in other tissues, thus augmenting the activation of the potassium conductance; however, we cannot exclude other possibilities, for example, that theophylline enhances the calcium sensitivity of the after-hyperpolarization potassium conductance.

Calcium-sensitive potassium conductances modulate the excitability and repetitive firing of neurons. Theophylline may therefore prove a useful tool for investigating regulation of neuronal activity and responsiveness.

Proposed Course:

This project will be terminated in NIMH due to reassignment of the P.I. to NIAAA.

Publications:

Neil A. Busis and Forrest F. Weight, Spike after-hyperpolarization of a sympathetic neuron is calcium-sensitive and is potentiated by theophylline. Nature, 263: 434-436, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 MH 01308-02 SMRN

PERIOD COVERED
Oct. 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Synaptic Transmission: Studies on the Physiology and Pharmacology of Post-synaptic Potential Generation

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Forrest F. Weight	M.D., Chief, Section on Synaptic Pharmacology	SMRN NIMH
	Peter A. Smith	Ph.D.	SMRN NIMH

COOPERATING UNITS (if any)

None

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NIMH, ADAMHA, NIH, St. Elizabeths Hospital, Washington, D.C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	0

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The ionic mechanisms involved in the generation of several types of post-synaptic potentials were investigated in the sympathetic ganglia of bullfrogs, by intracellular and sucrose gap recording techniques. The fast EPSP has a marked decreased in membrane resistance at its peak and has a reversal potential at -16mV. These data, together with ion change experiments, indicate that the fast EPSP is generated by an increase membrane conductance to both sodium and potassium ions. In contrast to this increased conductance mechanism, the slow postsynaptic potentials (the slow EPSP and the slow IPSP) are generated by different membrane mechanisms. Membrane resistance is increased during the slow EPSP, and membrane polarization shows that the slow EPSP reverses near the slow potassium equilibrium potential. We propose that the slow EPSP, in contrast to classical fast EPSPs, is generated by an inactivation of resting potassium conductance. The slow IPSP in these neurons is also associated with an increase in membrane resistance, and electrical polarization of the membrane indicates that the slow IPSP is nullified by a strong depolarizing current. We propose that the slow IPSP, in contrast with other types of fast IPSPs in the nervous system, is generated by an inactivation of resting sodium conductance.

Project Description:

Objectives:

Previous investigations in the vertebrate and invertebrate nervous system have shown that postsynaptic potential generation is due to an increase in ion conductance at virtually all chemically transmitting junctions which have been investigated. In sympathetic ganglia, slow postsynaptic potentials have been found with mechanisms which differ from those of other types of synaptic potentials in the central and peripheral nervous system. The objective of this study is to investigate the ionic membrane mechanism involved in the generation of these slow synaptic potentials, and their functional significance.

Methods Employed:

The ninth and tenth paravertebral sympathetic ganglia of the bullfrog were studied in vitro with continuous perfusion of oxygenated Ringer's solution. Intracellular records were taken from sympathetic ganglion cells using microelectrodes filled with 3 M KCl. Standard techniques of electrophysiological recording were used, current being injected through the recording microelectrode by means of a Wheatstone bridge circuit. Preganglionic afferents were stimulated in the sympathetic chain and the 8th spinal nerve. Ganglion cells were identified on the basis of antidromic conduction velocity.

Major Findings:

The fast EPSP, like most other EPSPs, was found to be due to an increase ion conductance. Hyperpolarization of the membrane increased fast EPSP amplitude, and depolarization decreased its size. Further depolarization then reversed the fast EPSP to a hyperpolarizing potential. The reversal potential was approximately -16 millivolts.

The electrophysiological properties of the slow EPSP differed significantly from those of the fast EPSP. Membrane resistance, measured by constant current pulses, increased significantly during the slow EPSP, indicating a decrease in membrane conductance. In contrast to the effect of membrane polarization on the fast EPSP, depolarizing current increased the size of the slow EPSP, whereas hyperpolarizing current decreased slow EPSP amplitude. Further hyperpolarization of the membrane reversed the slow EPSP to a hyperpolarizing potential. The reversal potential for the slow EPSP was near the potassium equilibrium potential.

The electrophysiological properties of the slow IPSP also differed significantly from the properties of synaptic inhibition at most synaptic junctions. Membrane resistance tested during the slow IPSP by constant current pulses indicate that membrane

resistance increased during the slow IPSP. The effect of membrane polarization also differed from the effect of polarization on increased conductance IPSPs. Moderate hyperpolarizing current increased the amplitude of the slow IPSP, whereas moderate depolarizing current decreased slow IPSP amplitude. Progressively stronger depolarizing current further decreased and then nullified the slow IPSP.

Significance to Bio-medical Research and the Program of the Institute:

The data in this investigation indicate that the fast EPSP, which is generated by the synaptic activation of nicotinic receptors, is due to an increase in sodium and potassium conductance of the membrane. This is similar to the mechanism of generation of most EPSPs in the vertebrate and invertebrate nervous system. On the basis of the data on the slow EPSP, we propose that the slow EPSP is generated by a decrease or inactivation of resting potassium conductance. This hypothesis provides an adequate explanation of the electrophysiologic properties of the slow EPSP demonstrated in this investigation. Similarly, to explain the properties of the slow IPSP found in this investigation, we propose that the slow IPSP is generated by a decrease or inactivation of resting sodium conductance. There are a number of suggestions that similar synaptic mechanisms are involved in slow postsynaptic potentials in the central nervous system; however, because of various technical and experimental limitations those mechanisms have not been adequately characterized. Thus, these mechanisms of synaptically inactivating resting membrane conductances may be of general significance in the synaptic control of neuronal function.

Proposed Course:

This project will be terminated in NIMH due to reassignment of the P.I. to NIAAA.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 01309-02 SMRN

PERIOD COVERED

October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Long-lasting Control of Postsynaptic Membrane Permeability and its Role in Controlling Synaptic Transmission Efficacy.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Forrest F. Weight M.D.,Chief, Section on Synaptic Pharmacology SMRN NIMH

Other: J. A. Schulman

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Neuropharmacology

SECTION

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	0

CHECK APPROPRIATE BOX(ES)

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Slow decreases of ionic conductance across neuronal cell membranes, which generate slow postsynaptic potentials, can increase the effectiveness of synaptic transmission. Slow conductance decreases of sufficient magnitude increase the amplitude of monosynaptic fast EPSPs in B cells of the bullfrog sympathetic ganglia. By this postsynaptic mechanism, activation of one synaptic pathway can cause an increase in transmission, lasting several minutes, across another synapse. This may provide an important mechanism for synaptic integration and control of neuronal interaction.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives:

An understanding of the mechanisms of synaptic transmission and their regulation may be important in the elucidation of the cellular mechanism underlying complex nervous function. The postsynaptic mechanisms by which EPSPs are generated and combined might provide a locus for control of synaptic function. However, the vast majority of known postsynaptic electrical responses are of short duration (tens of msec). Thus, postsynaptic interactions of such responses are necessarily brief and require a high degree of synchrony of convergent inputs. These interactions, therefore, do not provide a lasting modification of responsiveness. In addition, when fast synaptic potentials combine, the result is at most the algrebraic sum, and often is less. The object of the present experiments was to test the effect of synaptically activated conductance decreases on the effectiveness of synaptic transmission. In this way, we evaluated the role of postsynaptic membrane permeability in the control of synaptic function.

Methods Employed:

Synaptic potentials were recorded intracellularly in the ninth and tenth paravertebral sympathetic ganglia of bullfrogs, in vitro. Preganglionic axons were stimulated in the sympathetic chain and the eighth spinal nerve. Iontophoresis was used to administer methacholine directly to the neuron under investigation. Standard electrophysiological techniques were used, a bridge circuit being utilized to inject current intercellularly. The late slow EPSP was elicited by stimulating the eighth spinal nerve at a frequency of 50 Hz for 2 seconds.

Major Findings:

The slow EPSP, which is generated by a decrease in membrane potassium conductance, was mimicked by the iontophoretic administration of methacholine. The effect of this conductance decrease on synaptic transmission was studied by observing the effect of iontophoretically administered methacholine on fast EPSPs. It was found that fast EPSP amplitude increased with methacholine activated increases in membrane resistance of greater than 8 megohms. The duration of the fast EPSP also increased during the methacholine induced decrease in membrane conductance. In addition, these enlarged EPSPs often activated the postsynaptic generation of action potentials.

It is important to know whether such a postsynaptic potentiation of synaptic transmission can be elicited by stimulation

of physiological pathways. To test this possibility, we utilized a separate preganglionic pathway to generate a conductance decrease. Repetitive stimulation of the eighth spinal nerve produces a slow depolarization lasting many minutes, the late slow EPSP. Membrane conductance was found to be decreased during the late slow EPSP. As with the methacholine response, the amplitude of the fast EPSP increased during the late slow EPSP, and the increased amplitude of the fast EPSPs often initiated action potential generation. The increase in postsynaptic action potential generation. The increase in postsynaptic action potential generation by fast EPSPs could last for 2 or more minutes. For example, in one experiment, at the peak of the response, 5 out of 6 fast EPSPs elicited action potentials, compared to none in a control. This is a clear demonstration of the facilitation of transmission in one synaptic pathway by activation of another.

Significance to Bio-medical Research and the Program of the Institute:

This investigation indicates that different types of depolarization and membrane permeability changes have different consequences for neuronal interaction and synaptic transmission. That is, conductance increases and electrical depolarization of the membrane decrease synaptic potentials generated by a given excitatory input, whereas conductance decreases potentiate them. By augmenting, rather than diminishing, a synaptic input, a conductance decrease makes that input more effective the generation of action potentials.

This control of the efficacy of synaptic transmission may be important in the function of neurons of the central nervous system. Central neurons receive an abundance of converging inputs, and several types of central neurons have conductance-decrease responses. It is thus possible that increasing transmission in a synaptic pathway by a conductance-decrease response may have a function in information processing in the central nervous system. Further, lasting modification of responsiveness to a synaptic input might be a mechanism for storage of information at the cellular level. Long-term control of postsynaptic membrane properties might provide such a modification of responsiveness.

Proposed Course:

This project will be terminated in NIMH due to reassignment of the P.I. to NIAAA.

Publications:

Schulman, J.A. and Weight, F.F.: Synaptic transmission: long-lasting potentiation by a postsynaptic mechanism. Science, 194: 1437-1439, 1976.

Weight, F.F. and Schulman, J.A.: Long duration potentiation of synaptic transmission by synaptically and iontophoretically activated decreases in postsynaptic membrane conductance. In Symposium on "Iontophoresis and Transmitter Mechanisms in the Mammalian Central Nervous System", edited by Ryall and Kelly, 1978, Elsevier/North Holland Biomedical Press, pp 409-411.

Weight, F.F.: Modulation of synaptic excitability: Introduction: Federation Proceedings, in press, 1978.

Weight, F.F.: Long-lasting synaptic potentials and the modulation of synaptic excitability. Federation Proceedings, in press, 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 01310-02 SMRN

PERIOD COVERED
October 1, 1977 to September 30, 1978.

TITLE OF PROJECT (80 characters or less)
Biochemistry and Pharmacology of Cyclic Guanosine Monophosphate (Cyclic GMP) and Cyclic Adenosine Monophosphate (Cyclic AMP) Activation by Synaptic Stimulation.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Forrest F. Weight M.D., Chief, Section on Synaptic Pharmacology SMRN NIMH

Other: P. Greengard
G. Petzold
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COOPERATING UNITS (if any)
Department of Pharmacology, Yale University Medical School.

LAB/BRANCH
Laboratory of Neuropharmacology

SECTION
Synaptic Pharmacology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, St. Elizabeths Hospital, Washington, D.C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The stimulation of cholinergic preganglionic nerve fibers resulted in an increase in cyclic guanosine monophosphate (cyclic GMP) and cyclic adenosine monophosphate (Cyclic AMP) in the sympathetic ganglia of the bullfrog. When the release of synaptic transmitter was prevented by a high-magnesium/low-calcium Ringer's solution, stimulation of preganglionic nerve fibers did not increase either cyclic GMP or cyclic AMP content. Atropine, a muscarinic antagonist that blocks the generation of both the slow IPSP and the slow EPSP in this tissue, also blocked the increase in cyclic GMP caused by preganglionic stimulation, but not the increase in cyclic AMP. The data indicate that the increases in both cyclic GMP and cyclic AMP are associated with synaptic transmission.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives:

Cyclic GMP is distributed in a wide variety of tissues, but little is known about its functional role. The functional significance of cyclic GMP in the nervous system and particularly the possible relationship of this nucleotide to cholinergic transmission require further clarification. The sympathetic ganglion is well suited for an investigation of a possible role of cyclic GMP in cholinergic transmission, because the cholinergic preganglionic nerve fibers form synapses with the neurons in the ganglion. Furthermore, in the bullfrog sympathetic ganglion, muscarinic cholinergic mechanisms are involved in the generation of both the slow EPSP and the slow IPSP. Additionally, data are available on the ionic basis of these slow postsynaptic potentials. We therefore used these sympathetic ganglia to investigate whether cyclic nucleotide content can be altered by stimulation of the preganglionic nerve fibers.

Methods Employed:

The ninth and tenth paravertebral sympathetic ganglia were excised from bullfrogs and placed in a bath of oxygenated Ringer's solution, together with the corresponding ganglia from the contralateral side, which served as the unstimulated control. The preganglionic nerve fibers were stimulated at a frequency of 10 Hz for various periods of time, the postganglionic response being monitored throughout the period of stimulation. Immediately after stimulation, the stimulated ganglia were immersed in a boiling water bath for 3 minutes. The cyclic nucleotides content of the ganglia was subsequently determined by radioimmunoassay.

Major Findings:

Stimulation of preganglionic nerve fibers produced a significant increase in cyclic GMP content. Cyclic GMP was elevated by a period of stimulation as short as 6 seconds. The increase in cyclic GMP was rapid during the first 15 to 30 seconds of stimulation, reaching a maximum concentration approximately twice that of control. This elevated cyclic GMP content was maintained for at least 2 minutes, the longest period of stimulation tested. Cyclic AMP content was also increased by preganglionic stimulation; for example, stimulation for 2 minutes increased the cyclic AMP content to more than 700% of control.

The increase in cyclic GMP and cyclic AMP content that occurred with 2 minutes of stimulation in normal Ringer's solution was not observed in ganglia stimulated for the same period of time in a high-magnesium, low-calcium Ringer's solution. Since high-magnesium, low-calcium Ringer is known to prevent transmitter release from nerve endings in the sympathetic

ganglion, this result suggests that the increase in cyclic nucleotide content in the sympathetic ganglia is associated with synaptic transmission, rather than with impulse conduction along the preganglionic nerve fibers.

Since atropine is a competitive antagonist of acetylcholine at muscarinic postsynaptic receptors, and blocks the generation of both the slow IPSP and the slow EPSP in sympathetic ganglia, we used atropine to determine whether muscarinic receptors might be involved in the increase in cyclic nucleotide content following stimulation of preganglionic nerve fibers. In the presence of atropine, preganglionic stimulation did not produce a significant increase in cyclic GMP content. On the other hand, the increase of cyclic AMP was not significantly reduced in the presence of atropine.

Significance of Bio-medical Research and the Program of the Institute:

Previous investigations have shown that cyclic GMP in nervous tissues can be elevated by the administration of acetylcholine or muscarinic cholinergic agonist, but it has not been previously demonstrated that stimulation of synaptic pathways can change cyclic GMP content. The data in this study demonstrates that cyclic GMP content of nervous tissue can be significantly increased by stimulation of presynaptic cholinergic axons. The increase in cyclic GMP and in cyclic AMP was antagonized by high-magnesium, low-calcium concentration suggesting that the increase is associated with synaptic transmission. The selective antagonism by atropine of the increase in cyclic GMP suggests that the cyclic GMP increase involves the activation of muscarinic postsynaptic receptors. The data in the present investigation are consistent with the possibility that cyclic GMP may be involved in the postsynaptic action of acetylcholine of muscarinic cholinergic synapses. Further investigation is necessary to determine the postsynaptic receptors involved in the cyclic AMP activation.

Proposed Course:

A paper on this project is currently in preparation. The project will be terminated in NIMH due to reassignment of the P.I. to NIAAA.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER ZO1 MH 01311-02 SMRN

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)
Investigation of the Possible Involvement of Cyclic Nucleotides in Synaptic Potential Generation

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Forrest F. Weight	M.D., Chief, Section on Synaptic Pharmacology	SMRN NIMH
Others:	J. A. Schulman	Ph.D.	Salk Institute
	N. A. Busis	M.D.	SMRN NIMH
	P. A. Smith	Ph.D.	SMRN NIMH

COOPERATING UNITS (if any)
Salk Institute, La Jolla, California

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SECTION
Synaptic Pharmacology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, St. Elizabeths Hospital, Washington, D.C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.7	0.6	0.1

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The possibility that the cyclic nucleotides, cyclic AMP and cyclic GMP may mediate the generation of the slow synaptic potentials was investigated in sympathetic ganglia of the bullfrog, using the sucrose gap technique. The administration of cyclic GMP, cyclic AMP, and their derivatives had no significant effect on membrane potential. The administration of theophylline (5 mM), an inhibitor of phosphodiesterase - the catabolic enzyme for cyclic nucleotides, produced a depolarization of the membrane and an increase in membrane conductance. The muscarinic slow IPSP and the hyperpolarization produced by the administration of methacholine were potentiated by theophylline. The muscarinic slow EPSP and the depolarization produced by the administration of methacholine were reduced by theophylline. The noncholinergic late-slow EPSP was reduced by theophylline. In calcium-free Ringer, theophylline greatly enhanced the methacholine hyperpolarization; in addition, the methacholine depolarization was virtually abolished. These results raise the question of whether the cyclic nucleotides may have a functional role other than generation of slow postsynaptic potentials.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives:

In vertebrate sympathetic ganglia stimulation of preganglionic afferents evokes long-duration postsynaptic potentials, the slow IPSP and the slow EPSP. The mechanism of generation of these slow postsynaptic potentials is of considerable interest because these synaptic potentials are not associated with increases in membrane conductance, as occurs in the generation of most fast postsynaptic potentials, and their prolonged duration allows novel long-lasting mechanisms of synaptic integration and control of neuronal interaction. Additionally, postsynaptic potentials with apparently similar mechanisms of generation have been found in the vertebrate central nervous system.

Cyclic nucleotides have been proposed previously to function as intracellular second messengers mediating the generation of the slow postsynaptic potentials in sympathetic ganglia; the slow IPSP being mediated by cyclic AMP and the slow EPSP by cyclic GMP. This hypothesis is based on certain electrophysiological and biochemical evidence from rabbit and bovine sympathetic ganglia. The electrophysiological evidence from rabbit ganglia includes the alteration of resting membrane potential by the extracellular administration of cyclic nucleotides, and the potentiation of the slow IPSP by theophylline, an inhibitor of cyclic nucleotides phosphodiesterase that potentiates cyclic nucleotide dependent processes in many tissues.

The membrane mechanisms involved in the generation of both the slow EPSP and the slow IPSP have been most extensively investigated by electrophysiological techniques in the ninth and tenth paravertebral sympathetic ganglia of the bullfrog. In biochemical studies of bullfrog ganglia, stimulation of the preganglionic afferents have been found to result in an increase in both cyclic GMP and cyclic AMP, suggesting the possibility of cyclic nucleotide involvement in the generation of the slow postsynaptic potentials. To compliment these biochemical studies and to further extend electrophysiological investigations into the nature of slow potential generation, we have studied the effect of the administration of cyclic nucleotides and theophylline to these sympathetic ganglia.

Methods Employed:

The ninth or tenth paravertebral sympathetic ganglia of bullfrogs were excised and placed in a special plexiglas sucrose gap apparatus. The ganglion was continuously superfused with oxygenated Ringer's solution and the preganglionic nerves in the

sympathetic chain and the eighth spinal nerve were placed in a pool of liquid paraffin for stimulation. Recording was accomplished with two calomel electrodes, isotonic KCl. Standard electrophysiological techniques were used to record the potentials from the sucrose gap chambers.

Preganglionic stimulation was accomplished by delivering brief current pulses from isolated stimulators through bipolar platinum electrodes placed on the preganglionic afferents in the paraffin pool. The slow EPSP was elicited by repetitive stimulation of the sympathetic chain rostral to the seventh ganglion. The slow IPSP and the late slow EPSP were elicited by repetitive stimulation of the eighth spinal nerve.

Calcium-free Ringer's solution was prepared by omission of calcium chloride from the standard Ringer's solution. In most experiments either nicotine (30 uM) or d-tubocurarine 70 μM) were used to antagonize the fast EPSP and the nicotinic cholinergic actions of methacholine.

Major Findings:

The slow IPSP in the sympathetic ganglia was considerably and reversably potentiated by theophylline. THe slow IPSP was increased whether it was generated in the presence of nicotine or d-tubocurarine block. The predominant effect of theophylline was an enhancement of slow IPSP amplitude. Theophylline also increased the duration of the slow IPSP but to a lesser extent. The slow EPSP was also affected by theophylline; however, the predominant effect was a reduction in slow EPSP amplitude.

Repetitive stimulation of the eighth spinal nerve also elicits a slow depolarization in these ganglia, the late slow EPSP. Although the late slow EPSP is abolished by the removal of extracellular calcium ions, it is not blocked by atropine, nicotine, or d-tubocurarine, and thus is considered to be noncholinergic. In virtually all experiments, the late slow EPSP was reduced in amplitude by theophylline.

Theophylline may potentiate transmitter release from preganglionic nerve terminals and thus affect the amplitude of slow postsynaptic potentials. To control for this possibility, we use brief superfusions of the muscarinic agonist, methacholine (1mM), to mimic the generation of the slow IPSP and the slow EPSP. The methacholine hyperpolarization was considerably and reversably potentiated by theophylline, just as the slow IPSP was potentiated. The methacholine depolarization was affected similarly by theophylline, as compared to the slow EPSP. In most experiments, theophylline reversably reduced the amplitude of the methacholine depolarization.

In a separate investigation, we found that theophylline potentiates the spike after-hyperpolarization in these sympathetic neurons, and that this potentiation is abolished by the removal of extracellular calcium ions. We therefore studied whether theophylline could alter the methacholine response of these neurons in calcium-free Ringer. Since synaptic transmission is abolished in the absence of extracellular calcium ions, this part of the study was conducted on the methacholine responses. In the absence of extracellular calcium ions, theophylline resulted in a very large potentiation of the methacholine hyperpolarization. Furthermore, the methacholine depolarization was virtually abolished.

In addition to the effects of theophylline on the slow postsynaptic potentials, theophylline also produced a depolarization of membrane potential. This depolarization achieved a plateau in 30-60 minutes after the introduction of the drug and persisted as long as theophylline was superfused. Return to the theophylline-free Ringer was associated with a gradual repolarization over 1 hour or more. Theophylline depolarized ganglia whether they were superfused with normal Ringer or with Ringer containing nicotine or d-tubocurarine. In addition to the depolarization, the recorded base-line during theophylline became irregular, which may be related to spontaneous hyperpolarizations seen in individual neurons with intracellular recording. The theophylline depolarization was associated with an increase in membrane conductance.

Cyclic GMP and several derivatives (dibutyryl cyclic GMP, 8-benzylthio cyclic GMP, and parachlorophenylthio cyclic GMP), as well as cyclic AMP and derivatives (dibutyryl cyclic AMP and monobutyryl cyclic AMP) superfused at concentrations of 1 mM for one to several minutes had no significant effect on the resting membrane potential of these sympathetic neurons, whether these substances were superfused with normal Ringer, or with Ringer nicotine, d-tubocurarine or theophylline. These results are in agreement with other experiments in this laboratory, using intracellular recording, in which bath applications or iontophoresis of these cyclic nucleotides or their derivatives had no significant effect on membrane potential.

Papaveine (0.1 or 0.01 M) reduced or did not change the amplitude of both the slow IPSP and slow EPSP. Although a potentiation of the slow IPSP was observed following the removal of paparaine. In the presence of 0.01M papaveine, theophylline was still able to potentiate the hyperpolarizing part of the methacholine response.

Prostaglandin E1, 0.1M to 0.01 μM, caused a dose dependent depression of the slow IPSP, but not the methacholine hyperpolarization, indicating that this was a presynaptic action of the drug. Preliminary investigations suggest that both the slow EPSP and methacholine depolarization are reduced by this drug.

Significance to Bio-medical Research and the Program of the Institute:

Theophylline is well known as an inhibitor of cyclic nucleotide phosphodiesterases, and it has been used as a pharmacological probe for the involvement of cyclic nucleotides in a variety of cellular processes. In the present investigation, we have found that theophylline affects slow postsynaptic potential generation, muscarinic postsynaptic responses, resting membrane potential, and membrane conductance. The elucidation of the mechanisms of these actions and their interrelation with one another and with inhibition of ganglionic cyclic nucleotide phosphodiesterase may provide insight into the mechanism of slow synaptic potential generation and the role of cyclic nucleotides in neuronal function.

Theophylline potentiates cyclic nucleotide-dependent processes in many tissues. If production of the slow IPSP and the slow EPSP were each mediated by cyclic nucleotides, then one might expect theophylline to potentiate both responses. Also papaveine, a more potent phosphodiesterase inhibitor failed to potentiate either the slow IPSP or slow EPSP. Our data show that theophylline potentiated only the slow IPSP. Since theophylline has been found to potentiate the release of acetylcholine at the neuromuscular junction, such a presynaptic effect could account for the potentiation of slow IPSP. The potentiation of the methacholine hyperpolarization by theophylline in these experiments indicates that theophylline can potentiate muscarinic hyperpolarization by postsynaptic mechanisms. Separate investigations in this laboratory have demonstrated that preganglionic stimulation increases both cyclic GMP and cyclic AMP in these neurons, suggesting that these cyclic nucleotides might be involved in the generation of synaptic potentials. The potentiation of the slow IPSP observed in the present experiments by theophylline may be due to inhibition of phosphodiesterase, although other interpretations are also possible.

The effect of theophylline on the muscarinic slow EPSP and the methacholine depolarization in the present experiments was in most cases a reduction in the amplitude of the depolarization. These data do not support the possibility that the slow EPSP

is mediated by cyclic GMP. It should be noted, however, that the data also do not exclude a mediation by a cyclic nucleotide, since it is possible that another theophylline effect, such as the increase in membrane conductance, may prevent a potentiation from being observed.

In addition to inhibiting phosphodiesterase, theophylline can also affect cellular calcium metabolism. In a separate investigation in this laboratory, we have demonstrated a calcium sensitive component in the spike after-hyperpolarization and showed that this calcium-sensitive potassium conductance is potentiated by theophylline. A separate project has shown that cyclic nucleotides are not involved in this potentiation. Since theophylline potentiates both the spike after-hyperpolarization and the slow IPSP, it is of interest to compare these two hyperpolarizing potentials. The action potential after-hyperpolarization is due to an increase potassium conductance, and thus is decreased by hyperpolarizing currents. On the other hand, the slow IPSP is generated by a decrease in sodium conductance and thus is potentiated by moderate hyperpolarizing currents. On the other hand, the slow IPSP is generated by a decrease in sodium conductance and thus is potentiated by moderate hyperpolarizing current. Additionally, the theophylline-induced potentiation of the after-hyperpolarization is abolished by the removal of extracellular calcium. In contrast, the theophylline-induced potentiation of the methacholine hyperpolarization is not abolished by calcium-free Ringer, and, in fact, thus muscarinic hyperpolarization is potentiated. The possibility should be considered that the theophylline induced effects on slow synaptic potential generation may be due to effects of theophylline on cellular calcium metabolisms or other membrane actions rather than as inhibition of phosphodiesterase. This idea is further supported by the observation that theophylline can still potentiate the methacholine hyperpolarization in the presence of papaveine, (0.01M). i.e. the potentiation can still occur when phosphodiesterase is inhibited.

Prostaglandin E1 has been reported to activate adenylate cyclase, however, this drug failed to potentiate either the slow EPSP or the slow IPSP. This observation is also inconsistent with the role of cyclic nucleotides in their mediation.

In the present study, the administration of cyclic GMP, cyclic AMP, and several of their derivatives failed to demonstrate any consistent effect on resting membrane potential. These negative findings should be interpreted with caution, however, due to lack of knowledge of the uptake and metabolism of exogenous cyclic nucleotides in these tissues. Nevertheless,

these results raise the question of whether the increase in cyclic nucleotides produced by stimulation of presynaptic nerve fibers may play a role other than the generation of slow postsynaptic potentials.

Proposed Course:

A full paper on this investigation is in preparation and a paper entitled "Postsynaptic Potential Generation appears independent of synaptic elevation of cyclic nucleotides in sympathetic neurons" was recently submitted for publication. This project will be terminated in NIMH due to the reassignment of the P.I. to NIAAA.

Publications:

N.A. Busis, J.A. Schulman, P.A. Smith and F.F. Weight Do cyclic nucleotides mediate slow postsynaptic potentials in sympathetic ganglia? Brit. J. Pharmac. 62: 378-379 (1978).

N.A. Busis, F.F. Weight and P.A. Smith. Synaptic potentials in sympathetic ganglia: Are they mediated by cyclic nucleotides? Science 200: 1079-1081 (1978).

P.A. Smith, F.F. Weight, N.A. Busis, and J.A. Schulman. Cyclic nucleotides and slow postsynaptic potential generation in sympathetic ganglia of the bullfrog. Fed. Proc. 37, 524 (1978).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER ZO1 MH 01312-02 SMRN

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Physiology and Pharmacology of Electrogenic Sodium Pump Activation and Inhibition in Neurons

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Peter A. Smith	Ph.D.	SMRN NIMH
	Forrest F. Weight	M.D., Chief, Section on Synaptic Pharmacology	SMRN NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Neuropharmacology

SECTION
Synaptic Pharmacology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, St. Elizabeths Hospital, Washington, D.C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.3	0.3	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The physiology and pharmacology of the activation and inhibition of the electrogenic sodium pump was investigated in the sympathetic ganglia of bullfrogs, by the sucrose gap technique. The administration of nicotinic cholinergic agonists produces a brief depolarization that is followed by an after-hyperpolarization that is due to activation of the electrogenic sodium pump. This electrogenic pumping of sodium was rapidly inhibited by potassium-free Ringer. The after-hyperpolarization was also inhibited after one hour in ouabain (1 uM). Potassium-free Ringer resulted in an accumulation of intracellular sodium ions, so that return to normal Ringer solution resulted in hyperpolarization of the membrane due to activation of the electrogenic sodium pump. This hyperpolarization was also inhibited after one hour in ouabain (1 uM). These results indicate that the electrogenic sodium pump can be activated in two ways: following a nicotinic cholinergic depolarization; and by return from potassium-free Ringer to normal Ringer's solution. Furthermore, the electrogenic sodium pump can be inhibited by potassium-free Ringer and by ouabain.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives:

In many, if not all, neurons, the outward pumping of sodium is coupled to the inward transport of potassium, with more sodium extruded than potassium taken in, thus producing an outward movement of positive charge. This electrogenic pumping of sodium produces a hyperpolarization of the membrane, without a change in membrane permeability. The objective of the present experiments was to characterize the activation of the electrogenic sodium pump and its inhibition.

Methods Employed:

The experiments were carried out on the ninth or tenth paravertebral sympathetic ganglia of the bullfrog using the sucrose gap technique. The preparation was continuously superperfused with an oxygenated Ringer's solution containing a potassium concentration of 2 mM. In experiments using potassium-free Ringer, KCl was omitted without change of the other ions. Drugs were added to the superperfusing Ringer's solution.

Major Findings:

The administration of acetylcholine or other nicotinic cholinergic agonists produces a brief depolarization due to an increased conductance to sodium and potassium ions. This is followed by an after-hyperpolarization that results from the electrogenic extrusion of accumulated intracellular sodium ions. This nicotinic afterhyperpolarization was used to characterize inhibition of electrogenic sodium pump by ouabain and potassium-free Ringer. Potassium-free Ringer rapidly inhibited the after-hyperpolarization. The inhibition persisted as long as the preparation was bathed in the potassium-free Ringer, but it was rapidly reversible. Inhibition of the after-hyperpolarization by ouabain occured more slowly. The after-hyperpolarization was abolished after one hour in ouabain (1 uM). This effect was only slowly reversable with the restoration of the after-hyperpolarization taking 4 hrs or more. These results indicate that potassium-free Ringer and ouabain are effective inhibitors of the electrogenic sodium pump in this tissue.

Omission of calcium from the Ringer's solution also resulted in an apparent blockade of the acetylcholine afterhyperpolarization. However, the response was restored if 10mM magnesium was added to the calcium free Ringers. In addition, the calcium antagonist, cobalt (4mM) did not block the acetylcholine after-hyperpolarization. This indicated that the blockade of the response by Ca-free Ringer was probably associated with the fall in membrane resistance which occurs when the preparation is bathed in this medium. In this situation, the current produced

by the electrogenic pumping of sodium does not produce a significant potential across the lowered membrane resistance.

Following the return from potassium-free Ringer to normal Ringer's solution there was a hyperpolarization of the membrane. This hyperpolarization was also inhibited after one hour in ouabain (1 uM) and this inhibition was also slowly reversable. This indicates that the hyperpolarization is due to the electrogenic pumping of the sodium ions accumulated intracellularly during sodium pump inhibition with potassium-free Ringer.

Significance to Bio-medical Research and the Program of the Institute:

The electrogenic sodium pump is important in nervous tissue for maintaining ionic gradients necessary for the functioning of nerve cells. In this investigation, we demonstrated two means of activating the electrogenic sodium pump in order to study its properties and the ability of certain experimental procedures or pharmacologic agents to inhibit the pump. We have found that potassium-free Ringer and ouabain (1 uM) function as effective inhibitors of the electrogenic pumping of sodium ions in this tissue. Also if the membrane resistance is lowered as in a calcium free medium, the pump potential is not observed, presumably because the pump current produces an insignificant potential drop across the lowered resistance. These data may be of importance in attempting to understand the functional significance of the electrogenic sodium pump in the nervous system.

Proposed Course:

This project will be terminated in NIMH due to the expiration of Dr. Smith's visiting fellow status and the reassignment of Dr. Weight to NIAAA.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 01313-02 SMRN

PERIOD COVERED
October 1, 1977 to September 30, 1978.

TITLE OF PROJECT (80 characters or less)
Pharmacology of Electrogenic Sodium Pump Inhibitors on the Generation of Slow Synaptic Inhibition.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Peter A. Smith	Ph.D.	SMRN NIMH
	Forrest F. Weight	M.D., Chief, Section on Synaptic Pharmacology	SMRN NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Neuropharmacology

SECTION
Synaptic Pharmacology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, St. Elizabeths Hospital, Washington, D.C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.3	0.3	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In the present experiments, the possibility of a direct role of the electrogenic sodium pump in slow synaptic inhibition was tested. The experiments were carried out on the ninth or tenth paravertebral sympathetic ganglia of the bullfrog using the sucrose gap technique. We found that although electrogenic sodium pumping was inhibited by ouabain or potassium-free Ringer, the slow IPSP was not blocked by ouabain and was enhanced in potassium-free Ringer. These data are not consistent with the hypothesis that the slow IPSP results from an activation of the electrogenic sodium pump.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives:

The mechanism of synaptic transmission at most synapses involves an increase in the conductance of the postsynaptic membrane for certain ions. Certain slow postsynaptic inhibitions in both the central and peripheral nervous systems are, however, not generated in this way. It has previously been proposed that the slow inhibitory postsynaptic potential (IPSP) in sympathetic ganglia, and certain slow postsynaptic inhibitions in the central nervous system, are due to a unique mechanism, the synaptic activation of the electrogenic sodium pump. In the present experiments, the direct role of the electrogenic sodium pump in slow postsynaptic inhibition was tested.

Methods Employed:

The experiments were carried out on the ninth or tenth paravertebral sympathetic ganglia of the bullfrog using the sucrose gap technique. The preparation was continuously superperfused with an oxygenated Ringer's solution. In experiments using a potassium-free Ringer's solution, KCl was omitted without a change of the other ions. The slow IPSP was elicited by stimulating the eighth nerve at a frequency of 50 Hz for 2 seconds. Drugs were administered by addition to the superperfusing of Ringer's solution.

Major Findings:

The application of potassium-free Ringer's solution produced little or no change in resting membrane potential, but resulted in a rapid and persistent increase in the amplitude of the slow IPSP. This increase in slow IPSP amplitude persisted as long as the preparation was perfused with potassium-free Ringer. To investigate the muscarinic slow IPSP, the nicotinic fast EPSP must be antagonized. When nicotine was used to antagonize the fast EPSP, 1 hour in ouabain (1 microM) resulted in a partial reduction in slow IPSP amplitude. When d-tubocurarine was used as a nicotinic antagonist, however, no significant reduction was observed after 1 hour in ouabain. Even after 90 minutes in ouabain, the administration of potassium-free Ringer still increased the amplitude of the slow IPSP.

The effect of ouabain and K-free on the response of the ganglion cells to methacholine was also examined. This muscarinic agonist activates directly the postsynaptic receptors involved in the generation of both the slow ipsp and the slow EPSP. In K-free Ringer, both the hyperpolarizing phase and the depolarizing phase of the methacholine response were enhanced. In curare blocked preparations, 1 μM ouabain potentiated both the

hyperpolarizing and depolarizing phases of the methacholine response. In nicotine blocked preparations, ouabain tended to depress or did not change the methacholine hyperpolarization; the depolarization was also little affected.

Calcium free Ringer, which lowers the membrane resistance so that the electrogenic pumping of sodium produces no apparent change in membrane potential, did not block the methacholine hyperpolarization.

Significance to Bio-medical Research and the Program of the Institute:

The present experiments provide information on the mechanism of generation of slow postsynaptic inhibition. It has been proposed that the slow IPSP is due to an activation of the electrogenic sodium pump. The present experiment show that although potassium-free Ringer and ouabain inhibit the electrogenic pumping of sodium, they do not block the slow IPSP. Since the methacholine response is in fact potentiated by ouabain it appears that the depression of slow IPSP observed may be due to a presynaptic action of this drug. This suggests that the slow IPSP is not due to the synaptic activation of the electrogenic sodium pump. Another hypothesis proposes that the slow IPSP is generated by a decrease in resting sodium conductance. The present results can be readily explained by and support this hypothesis. The mechanism of generation of the slow IPSP may be important for information processing in the nervous system.

Proposed Course:

A paper on this project is in preparation for publication. The project will be terminated in NIMH due to the expiration of Dr. Smith's visiting fellow status and the reassignment of Dr. Weight to NIAAA.

Publications:

Smith, P.A. and Weight, F.F.: Role of electrogenic sodium pump in slow synaptic inhibition is re-evaluated. Nature, 267: 68-70, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 01314-02 SMRN

PERIOD COVERED
Oct. 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Lithium: Physiology and Pharmacology of Effects on Neuronal Membrane and Synaptic Potential Generation

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Peter A. Smith	Ph.D.	SMRN NIMH
	Forrest F. Weight	M.D., Chief, Section on Synaptic Pharmacology	SMRN NIMH
	Gerald A. Campbell	M.D., Ph.D.	SMRN NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Neuropharmacology

SECTION
Synaptic Pharmacology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, St. Elizabeths Hospital, Washington, D. C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.3	0.3	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The cellular mechanisms of the action of lithium in the nervous system is of interest because of its importance in treating certain types of mental illness. We tested the effect of lithium on the electrogenic sodium pump and the slow IPSP in bullfrog sympathetic ganglia, using the sucrose gap technique. The administration of acetylcholine (3 mM) produces a nicotinic depolarization followed by an after-hyperpolarization that results from the activation of the electrogenic sodium pump. When sodium in the Ringer's solution was replaced by lithium, the acetylcholine depolarization still occurred but the after-hyperpolarization was abolished. Presumably lithium is not extruded by the electrogenic sodium pump, as has been found previously in other tissues. Lithium Ringer also blocked the muscarinic slow IPSP and the hyperpolarizing response to agonists such as methacholine. We have recently found that ouabain and potassium-free Ringer can effectively inhibit the electrogenic sodium pump without blocking the slow IPSP, indicating that the slow IPSP is not generated by activation of the electrogenic sodium pump. In view of this, we conclude that the effect of lithium on the slow IPSP is not a direct consequence of sodium pump inhibition.

Project Description:

Objectives:

The cellular mechanisms of action of lithium are of particular interest because of its importance in treating manic-depressive psychosis. The objective of the present experiments was to study the effect of lithium on membrane permeability, on the electrogenic sodium pump, and on various electrogenic processes in neuronal membrane, including the generation of synaptic potentials.

Methods Employed:

In this study, the effects of lithium were investigated on the ninth and tenth paravertebral sympathetic ganglia of the bullfrog, in vitro. The sucrose gap technique was used to record changes in membrane potential and the generation of electrogenic processes. The ganglion was continuously superfused with oxygenated Ringer's solution. The effect of lithium was investigated by substituting lithium ions for sodium ions in the Ringer's solution. Drugs were administered by addition to the superfusing Ringer's solution.

Major Findings:

The administration of lithium Ringer resulted in a hyperpolarization of the membrane potential of the sympathetic ganglion neurons. To determine the possible ionic basis of this membrane hyperpolarization, electrical resistance of the membrane was tested with constant current pulses. It was found that membrane resistance increased the lithium Ringer. This indicates that membrane conductance was decreased in the lithium neurons than sodium conductance.

The effect of lithium was also tested on the generation of synaptic potentials. In addition to hyperpolarizing the membrane potential, lithium Ringer was found to markedly reduce or abolish the generation of the slow IPSP. Although there were some changes in the fast EPSP and the slow EPSP, both of these synaptic potentials were generated in the presence of the lithium Ringer's solution. Since the effects of lithium on the slow IPSP might be due to presynaptic actions of lithium, the effect of lithium was also tested on the hyperpolarization produced by the administration of the muscarinic agonist, methacholine, which directly activates muscarinic postsynaptic receptors to mimic the slow IPSP. As was found with the effect of lithium on the slow IPSP, lithium abolished the hyperpolarization produced by the administration of methacholine. The depolarizing phase of the main response, however, appeared to be enhanced by lithium.

In addition to these cellular actions, lithium was also found to substitute extracellularly for potassium ions in

activation of the electrogenic sodium pump. If the ganglia are treated with potassium-free Ringer's solution, there is a resultant accumulation of intracellular sodium ions. When extracellular potassium ions are restored by superfusion with a normal Ringer's solution, the electrogenic sodium pump is reactivated and a membrane hyperpolarization results due to the electrogenic pumping of sodium ions. The substitution of 15 mM lithium Ringer for potassium-free Ringer also results in a reactivation of the electrogenic sodium pump.

Significance of Bio-medical Research and the Program of the Institute:

In this investigation, lithium Ringer's solution has been found to produce a hyperpolarization of the neurons and a decrease or abolition of the slow IPSP and the methacholine hyperpolarization. The reduction in slow IPSP amplitude and the amplitude of the methacholine hyperpolarization would not appear to be due to the hyperpolarization of the membrane produced by lithium, because electrical hyperpolarization of the membrane results in a potentiation of both the slow IPSP and the methacholine hyperpolarization. In a separate investigation, we have found that ouabain and potassium-free Ringer can effectively inhibit the electrogenic sodium pump in these neurons, without blocking the slow IPSP or the methacholine hyperpolarization, indicating that the slow IPSP is not generated by activation of the electrogenic sodium pump. In view of this, we conclude that the effect of lithium on the slow IPSP is not consequence of sodium pump inhibition.

The administration of lithium Ringer produced not only a hyperpolarization of the neuronal membrane potential, but also an increase in membrane resistance. This indicates that membrane conductance was decreased in lithium Ringer and suggests that the resting permeability of the membrane to lithium is less than that to sodium ions. Since the slow IPSP is thought to be due to be generated by a decrease in resting sodium conductance, a low resting lithium conductance may account for the reduction in the amplitudes of the slow IPSP and methacholine hyperpolarization. On the other hand, it is known from previous investigations that lithium ions are not extruded by the electrogenic sodium pump. Therefore, it is also possible that the reduction in slow IPSP and methacholine hyperpolarization amplitudes may be due to an intracellular accumulation of lithium ions. Thus, the mechanism of the lithium inhibition of the slow IPSP is, at present, uncertain, and is currently the subject of active investigation.

Proposed Course:

A paper on this project entitled "Lithium: effects on the electrogenic sodium pump and slow synaptic inhibition" was presented at the Society for Neuroscience Meeting in November, 1977. The project will be terminated in NIMH due to expiration of Dr. Smith's visiting fellow status and the reassignment of the other investigators to NIAAA.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 01318-01 SMRN

PERIOD COVERED
October 1, 1977 to September 30, 1978.

TITLE OF PROJECT (80 characters or less)
Dopamine and Haloperidol Actions on Central Neurons.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Gerald A. Campbell	Staff Fellow	SMRN NIMH
Other:	Forrest F. Weight	Chief, Section on Synaptic Pharmacology	SMRN NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Neuropharmacology

SECTION
Synaptic Pharmacology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, St. Elizabeths Hospital, Washington, D.C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.3	1.2	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The technique of microiontophoresis was utilized to study the effect of dopamine administration on the activity of neurons in the region of the subthalamic nucleus. Five-barrel microelectrodes were placed stereotaxically in the subthalamic nucleus in urethane-anesthetized rats and dopamine was applied iontophoretically to both spontaneously active cells and to cells whose activity was maintained by continuous iontophoretic application of glutamate. Acetylcholine and haloperidol were also applied to some cells. Both increases and decreases in the spontaneous firing of neurons in the vicinity of the subthalamic nucleus were observed. In addition, some of these responses could be blocked by haloperidol. Relatively few responses (increases or decreases in firing rate) to acetylcholine were observed.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives:

Recent studies using glucose utilization measures by the ^{14}C-deoxy-D-glucose technique as an index of neuronal activity have indicated that the activity of subthalamic nucleus neurons is increased by a greater amount than most other areas of the brain by apomorphine, a dopamine agonist, and d-amphetamine, which enhances release and reuptake of catecholamines. In view of these results and because of the established importance of dopamine in the extrapyramidal system, we used microiontophoresis to assess the effects of dopamine administration on the activity of single neurons in the subthalamic region. In addition, acetylcholine, also known to be important in the extrapyramidal system, and haloperidol, a neuroleptic known to block dopamine receptors, have been administered microiontophoretically. As an integral part of this project it was necessary to develop a systematic technique for accurate stereotaxic placement of microelectrodes and marking of tip positions.

Methods Employed:

In this study, 5-barrel micropipettes were used to simultaneously record extracellularly from single neurons and inject drugs iontophoretically into the extracellular space near these neurons. Currents of 10-100 neuroamperes used to inject drugs from the micropipettes were generated by a 4-channel constant current source which provided a balance current equal to the sum of the currents in the three drug channels and opposite in polarity. The balance current was passed through 3M saline solution in one barrel of the micropipette to minimize the effects of current passage on theneurons under study. Conventional electrophyiological recording techniques were employed, and observed firing rates were displayed as ratiometric tracings on chart recorder paper and in some cases as an average of many responses calculated by a PDP-12 computer.

Male Sprague-Dawley rats (140-160 gm), anesthetized with urethane (25% in normal saline, 6 ml/kg) were used for this study. The cortex was removed by suction around the insertion site of the microelectrode down to the dorsal surface of the from hippocampus to provide a landmark which the depth of penetration could be accurately measured. In addition, horseradish peroxidase (Sigma type VI, 3% in 5M saline) was injected iontophoretically from the recording barrel at the point of maximal penetration to further mark the recording site. Following each experiment the animal was perfused with a wash solution and fixative, and the brain was cut in 40 micron frozen sections. A tetramethylbenzidene technique was then employed to visualize the peroxidase.

Major Findings:

Many of the cells investigated in the vicinity of the subthalamic nucleus responded to dopamine administration, while relatively few responded to administration of acetylcholine. Both increases and decreases in spontaneous firing rate in response to dopamine administration were recorded from neurons in the same animals with the same microelectrode. Some but not all of the dopamine responses were blocked by haloperidol.

Significance to Biomedical Research and the Program of the Institute:

The significance of dopamine in the nigro-striatal pathway and its role in Parkinson's disease are now well-established. It is also important to investigate the possibility that dopamine may act on neuronal receptors in other parts of the extrapyramidal system. This possibility for the subthalamic nucleus is strengthened by the glucose utilization studies and by the recent clinical observations that hemiballismus, an extrapyramidal movement disorder in man, which may result from lesions of the subthalamic nucleus or its connecting pathways, can often be controlled by neuroleptics such as haloperidol. The present studies support the hypothesis that dopamine acts on neuronal receptors in the subthalamic nucleus.

Proposed Course:

A paper on this project entitled "Action of Microiontophoretic Administration of Dopamine on Subthalamic Nucleus Neurons in the Rat" will be presented at the Society for Neuroscience Meeting in November, 1978. This project will be terminated in NIMH due to reassignment of the investigators to NIAAA.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 01319-01 SMRN

PERIOD COVERED
October 1, 1977 to September 30, 1978.

TITLE OF PROJECT (80 characters or less)
The Role of Cyclic Nucleotides in the actions of Theophylline on Neuronal Membranes.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.:	Peter A. Smith	Ph.D.	SMRN NIMH
	Richard Lehne	Ph.D.	
	Forrest F. Weight	M.D., Chief, Section on Synaptic Pharmacology	SMRN NIMH
	T. W. Rall	Ph.D.	

COOPERATING UNITS (if any)
Department of Pharmacology, University of Virginia, Charlottesville, Virginia.

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SECTION
Synaptic Pharmacology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, St. Elizabeths Hospital, Washington, D.C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Theophylline inhibits cyclic nucleotide phosphodiesterase and potentiates cyclic nucleotide dependent processes in many tissues. This methylxanthine greatly prolongs the afterhyperpolarization of a directly evoked action potential in bullfrog sympathetic ganglion cells. Cyclic nucleotides could therefore be involved in this process. This possibility was investigated by means of the sucrose gap technique. Theophylline increased the concentration of cyclic AMP and cyclic GMP in these ganglia. However a variety of other agents which elevated cyclic nucleotides in these ganglia did not augment the spike after-hyperpolarization. Application of high doses of cyclic nucleotides did not affect the spike afterhyperpolarization. Additionally, following inhibition of phosphodiesterase by 10^{-4}M papaverine, theophylline still potentiated the spike afterhyperpolarization. It therefore appears that the mechanism whereby theophylline potentiates the spike afterhyperpolarization is independent of the cyclic nucleotides.

Project Description:

Objectives:

Theophylline potentiates cyclic nucleotide dependent processes in many tissues and is known to inhibit phosphodiesterase, the enzyme which inactivates cyclic AMP and cyclic GMP. Theophylline also greatly prolongs the after-hyperpolarization of a directly evoked action potential in bullfrog sympathetic ganglion cells. The possible role of cyclic nucleotides in this phenomenon was therefore investigated.

Methods Employed:

Electrophysiological experiments were conducted on the ninth and tenth paravertebral sympathetic ganglion of the bullfrog by means of the sucrose gap technique. The ganglia were continuously superfused with an oxygenated Ringers solution. The action potential afterhyperpolarization was evoked by passing a 2 msec pulse of positive current across a bridge circuit. To measure cyclic nucleotide concentrations, ganglia were fixed in liquid nitrogen and the nucleotides extracted in cold methanol/hydrochloric acid prior to assay by a radioimmunological method.

Major Findings:

Theophylline (5mM) elevated the concentrations of cyclic GMP and cyclic AMP in the ganglia and caused a marked prolongation of the action potential afterhyperpolarization. However, the time-course of the action of theophylline on the nucleotides was different from its time-course on the action potential afterhyperpolarization. Papaverine (0.1mM), Methacholine (1mM), Diazepam (0.1mM) or synaptic stimulation also elevated the levels of cyclic nucleotides but did not enhance the afterhyperpolarization. Addition of high concentrations of cyclic AMP or cyclic GMP (3mM) did not mimic the effect of theophylline on the action potential afterhyperpolarization. It was therefore concluded that theophylline exerted its effect on the afterhyperpolarization by some means other than by the inhibition of phosphodiesterase.

Significance to Bio-medical Research and the Program of the Institute:

The cyclic nucleotides, cyclic AMP and cyclic GMP, have been suggested to act as second messengers in the generation of various synaptic potentials. Theophylline is known to potentiate the action potential afterhyperpolarization in sympathetic ganglia as well as certain synaptic potentials in a variety of systems. Theophylline inhibits phosphodiesterase and thus the breakdown of cyclic nucleotides. Thus potentiation of an electrophysiological phenomenon by this drug has hitherto been

taken as evidence that cyclic nucleotides are involved in the production of that phenomenon. The present data illustrate that theophylline can have effects independent of its action as a phosphodiesterase inhibitor. This illustrates that theophylline, a drug frequently assumed to be a specific phosphodiesterase inhibitor, can act by mechanisms independent of inhibition of phosphodiesterase.

Proposed Course:

A paper reporting this project is currently in preparation. The project will be terminated in NIMH due to reassignment of the P.I. by NIAAA.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 01320-01 SMRN

PERIOD COVERED
Oct. 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)
Synaptic Pathways in Hippocampal Cortex, in vitro.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.:	Herbert J. Doller	Staff Fellow	SMRN NIMH
Other:	Forrest F. Weight	M.D., Chief, Section on Synaptic Pharmacology	SMRN NIMH

COOPERATING UNITS (if any)
None

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SECTION
Synaptic Pharmacology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, St. Elizabeths Hospital, Washington, D.C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	0.9	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Hippocampal cortex from guinea pigs was studied, in vitro, by electrophysiological techniques. Slices of hippocampus approximately 0.3-0.4 mM thick permit visualization of neuronal cell layers and synaptic pathways. Various synaptic pathways have been identified by electrical stimulation and by recording in neuronal cell layers in different regions of the hippocampus. Changes in ionic composition of the superfusing solution have been used to characterize the synaptic responses. These synaptic pathways are being used to study the nature of the synaptic transmitter released and the interaction of psychotrophic and other centrally acting drugs. In addition, a long-term potentiation of synaptic transmission is also the subject of current investigation.

PHS-6040
(Rev. 10-76)

Project Description:

Objective:

A hippocampal pathway originating in the entorhinal cortex, traversing as part of the perforant pathway and terminating on the apical dendrites of the CA1 pyramidal cells has been anatomically described. Electrophysiolical identification and characterization, however, has remained inconclusive. We used the hippocampal slice technique to study of this pathway. Since neuronal cell layers can be visualized, stimulating and recording electrodes can be accurately placed. In addition, lesions can be made accurately in specific areas of the hippocampus.

Methods Employed:

After sacrificing the animal, the hippocampus was quickly dissected from the brain and placed in iced Lockes solution. The hippocampus was then sliced in tranverse sections 0.3-0.4 mM thick, placed in a recording chamber at 37°C. Because the major intrinsic pathways of the hippocampus lie in plane of section, they can be studied in the slice. The laminar structure of the neuronal cell layers of the hippocampus is clearly visable under a microscope. Recording electrodes can thus be visually placed in the desired cell layers and stimulating electrodes can be placed on the pathways. Evoked potentials were recorded from glass electrode having a tip diameter of 3-8 µM, filled with 3 M NaCl, and having a resistance of 1.5-3 megohms.

Major Findings:

When a stimulating electrode was placed in the perforant pathway, an evoked potential with a latency of approximately 10 msec was recorded in the CA1 cell body layer. This response was inhibited by media containing 0.1 mM Ca and 5 mM Mg , suggesting that the field potential was synaptically mediated. Two types of experiments were performed to determine whether this CA1 response was the result of activation via the mossy fibers and Schaeffer collaterals from granule cells to CA3 to CA1. The first experiment approximated the latency of this multisynaptic pathway. This was accomplished by placing a second recording electrode in the granule cell body layer and a second stimulating electrode in the stratum lucidum of the CA3 pyramidal cells. The latency of the perforant stimulating electrode to granule cells was determined by stimulating the perforant pathway and recording in the granule cell body layer. The latency between

the granule cells and the CA3 cells was approximated by stimulating the stratum lucidum in the CA3 region and recording evoked responses in the granule cell layer. This response was insensitive to the low CA high Mg media, thus supporting the premise that this response represented an antidromic activation of the granule cells. Stimulating with the same electrode and recording in the CA1 cell body layer, the CA3-CA1 latency was determined. Thus the latencies of all pathways could be approximated in a single slice. The sum of the latencies representing the perforant stimulating electrode to granule cells to CA3 cells to CA1 cells was about twice that of the perforant stimulating electrode directly to the CA1 cell body layer. In a second series of experiments the mossy fiber pathway which connects the granule cells to the CA3 cells was transected. Despite interruption of the mossy fiber pathway, field potentials from stimulating the perforant pathway could still be recorded in the CA1 cell body layer. It is also possible that Schaeffer collaterals, which connect the CA3 cells to the CA1 cells are being antidromically stimulated by the electrode in the perforant pathway. To test for this possibility, 1 mm sections were cut out of the CA1 region between the perforant stimulating electrode and the CA1 recording electrode. The tissue removed included a section of the alveus down to but not including the perforant pathway (stratum molecular). Field potentials from stimulating the perforant pathway were still recorded in the CA1 cell body layer after removal of the region of CA1 which contains the Schaeffer collateral axons. These results provide electrophysiological evidence for the existence of a pathway from the entorhinal cortex via the perforant pathway to the CA1 pyramidal cells.

Significance to Biomedical Research and the Program of the Institute:

Hippocampal cortex is a region of the central nervous system that has been implicated in learning and behavior. By studying the synaptic pathways and the responses of neurons in this cortical region, we may learn about the mechanisms of central synaptic function. Additionally, these synaptic pathways are being used to study the central mechanisms of psychotrophic drug action and the mechanisms of long-lasting potentiation of synaptic transmission.

Proposed Course:

A paper on this project entitled "Electrophysiological Evidence for the Perforant-CA1 Pathway in the Hippocampal Slice" will be presented at the Society for Neuroscience Meeting in November, 1978. This project will be terminated in NIMH due to the reassignment of the investigators to NIAAA.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 01481-05-SMRP

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)
Short and long term regulation of tryptophan hydroxylase in brain

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. L. Meek	Pharmacologist	SMRP NIMH
Other:	E. Marco	Visiting Fellow	SMRP NIMH

COOPERATING UNITS (if any)
None

LAB/BRANCH
Laboratory of Preclinical Pharmacology

SECTION
Molecular Neurobiology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, St. Elizabeths Hosp., Washington, D. C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Serotonin turnover was studied in discrete brain areas of rats treated with various antidepressants. Changes in turnover were estimated using the probenecid, pargyline and decarboxylase inhibitor methods. Chlorimipramine decreased serotonin turnover in the hypothalamus, hippocampus, cortex, septum and nucleus caudatus in doses as low as 1.8 mg/kg, a dose similar to that used in man. This effect on turnover was seen after another drug (fluoxetine) that affects 5HT reuptake, but not after antidepressants that do not affect 5HT reuptake (desmethylimipramine, amitryptiline, iprindole or amphetamine).

PHS-6040
(Rev. 10-76)

Project Description:

A major hypothesis as to the mechanism of action of antidepressive drugs postulates that they influence the amine reuptake pump located in the membrane of aminergic neurons. As a consequence, the drugs decrease the firing rate of the neurons, and decrease the sythesis and turnover of the amines. Some antidepressants (e.g. desmethylimipramine) are known to affect norepinephrine uptake, other antidepressants (e.g. chlroimipramine) are known to affect primarily 5HT uptake while still others, e.g. iprindole do not affect either system. No currently known mechanism is common to all of the clinically effective antidepressants. However, effects on various amine neurons, which occur immediately, may ultimately result in the same clinical improvement seen after weeks or months. In any case, study of turnover is probably the clearest indication now available that a drug is active in vivo, and that it affects the firing of an amine neurons. However, studies to date on turnover have been carried out only in whole brain due to the lack of sufficiently sensitive methodology to analyze individual pathways. The development of high pressure liquid chromatographic (HPLC) techniques of great sensitivity in this lab for assay of hydroxyindoles now permits assessment of possible differential anatomical action of drugs. We can therefore begin to ask questions about whether drugs act uniformly throughout the brain or whether effects are restricted to areas innervated by one nucleus e.g. the median, but not dorsal raphe. We have therefore examined in finer anatomical detail the action of these antidepressants. The OBJECTIVES of this project are to 1) determine whether chlorimipramine uniformly affects 5HT turnover in all areas of brain. 2) to determine whether antidepressant drugs which do not alter whole brain 5HT turnover might owe their efficacy to a selective action on 5HT neurons in certain areas.

The methods employed included measurement by HPLC of serotonin, 5 hydroxytryptophan, and 5 hydroxyindoleacetic acid in discrete brain areas of controls and rats treated with the antidepressants. The rats were pretreated with pargyline or a decarboxylase inhibitor or probenecid to alter the steady state concentrations of the hydroxyindoles and allow an estimation of turnover.

Major Findings

1. Chlorimipramine in low doses (1.8 mg/kg) decreased serotonin turnover in all areas tested: hypothalamus, hippocampus, cortex, septum and nucleus caudatus.

2. Fluoxetine, which like chlorimipramine specifically blocks 5HT uptake also decreased 5HT turnover.

3. Antidepressant drugs which affect the catecholaminergic but not serotonergic systems did not change 5HT turnover in any of the areas tested (desmethylimipramine, amitryptiline, iprindole, amphetamine). The significance of this work lies in its providing tools for dissecting out functional difference between various amine pathways, and highlighting areas that need to be studied. For example, existing knowledge of regulation of aminergic neurons firing is so rudimentary, that it is not known whether inhibition of firing via a negative neuronal feedback loop affects all nuclei if only the receptors in one area had increased stimulation. This question could be studied by finding whether local unilateral injections of antidepressant drugs resulted in local effects on turnover. These experiments will be carried out by 2 investigators (Drs. Marco and Neckers) who received training in HPLC in this lab, and have now gone on to work elsewhere. A description of the above experiments with the antidepressants has been submitted to Archives of Pharmacology.

This project has been terminated.

Publications:

Meek, J. L. and Neckers, L. M.: Studies of serotonin turnover in discrete nuclei using HPLC. In Usdin, E. Weiner, N., and Youdim, N. H. B. (Eds.): Structure and Function of Monoamine Enzymes. New York, Marcel Dekker, 1977, pp. 799-809.

Meek, J. L., Bertilsson, L., Cheney, D. L., Zsilla, G., and Costa, E.: Aging induced changes in acetylcholine and serotonin content of discrete brain nuclei. Journal of Gerontology 32: 129-131, 1977.

Meek, J. L.: Studies on serotonin neurotoxins in discrete brain nuclei. Annals N. Y. Academy of Sciences, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01487-03 SMRP

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Regulation of phosphodiesterase by a protein activator

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. E. Gnegy	Staff Fellow	SMRP	NIMH
Other:	P. Uzunov	Visiting Scientist	SMRP	NIMH
	E. Costa	Chief		

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Preclinical Pharmacology

SECTION
Molecular Neurobiology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, St. Elizabeths Hosp., Washington, D.C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This work extends the study of the mechanism of phosphodiesterase regulation by an endogenous protein activator. The activator stored in the cell membrane fraction can be released into the cytosol by a cAMP-dependent phosphorylation of a membrane binding site.

A subcellular distribution of the localization of activator in rat brain and its release has been performed. The activator is located mainly in the mitochondrial fraction and particularly in the mitochondrial subfractions containing synaptic membranes and synaptic vesicles. Nearly 100% of the activator in these fractions can be released by the cAMP-dependent phosphorylation. The mechanism for cAMP during periods of sustained synaptic transmission and could explain post-synaptic receptor supersensitivity to neurotransmitters.

PHS-6040
(Rev. 10-76)

This project has been terminated.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NO.

Z01 MH 01489-05 SMRP

PERIOD COVERED

October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Regulation of gene expression in neuroblastoma

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. A. Hollenbeck	Pharmacologist	SMRP	NIMH
Other:	D. M. Chuang	Chemist	SMRP	NIMH
	E. Costa	Chief	SMRP	NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Preclinical Pharmacology

SECTION

Molecular Neurobiology

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, St. Elizabeths Hosp., Washington, D.C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

We have found that activation of protein kinase by 8 bromo adenosine 3',5'-cyclic monophosphate (8 Br-cAMP) in cultured neuroblastoma cells (NB) is followed by preferential phosphorylation of nuclear nonhistone chromosomal proteins and the induction of tyrosine hydroxylase (TH). Our experimental results suggest that TH induction in NB may be preceded by a modulation of gene expression through translocation of protein kinase from cytosol to nucleus and phosphorylation of nuclear nonhistone protein.

PHS-6040
(Rev. 10-76)

Project Description:

In the chromaffin cells of adrenal medulla, the transsynaptic induction of tyrosine hydroxylase is caused by persistent stimulation of acetylcholine receptors and is mediated by activation of the cyclic AMP-dependent protein kinase of cytosol. This activation is followed by the translocation of catalytic subunits of this cyclic AMP-dependent protein kinase from cytosol to the nucleus. Since evidence exists which suggests that phosphorylation of nuclear nonhistone protein is involved in gene control mechanisms, we decided to try to elucidate which nuclear protein may be specifically phosphorylated during tyrosine hydroxylase induction. Our experiments would involve the use of cultured neuroblastoma cells (line NBD-2) which constitutes an in vivo system possessing catecholamine biosynthetic enzymes and which is more amenable to direct phosphorylation study than our previous model, the adrenal medulla.

A series of experiments was planned to increased our understanding of the mechanisms that regulate communication between membrane receptors and molecular neuroblastoma. The results support that: 1) a cyclic AMP analogue, 8 Br-cAMP, induces tyrosine hydroxylase of NBD_2 when added for about 30 min to the cell culture under our experimental condition; 2) protein kinase is activated in neuroblastoma by addition of 8 Br-cAMP and 3) protein kinase is translocated from cytosol to the nucleus following the addition of 8 Br-cAMP.

We found that when cells grown in monolayer were exposed to 8 Br-cAMP for as little as 15 minutes, protein kinase activity was maximally activated within a hour with 8 Br-cAMP concentration as low as 0.2 mM. Identical treatment of the cells produced up to a 100% increase in tyrosine hydroxylase activity measured 48 hours after exposure to the nucleotide.

Since earlier experiments with adrenal medulla had shown that protein kinase activation by cyclic AMP preceded translocation and subsequent tyrosine hydroxylase induction, we measured the time course of protein kinase activation in neuroblastoma and the subcellular distribution of protein kinase activity after exposure of the cells for 15 minutes to 8 Br-cAMP. We found that activation of protein kinase in whole cell homogenates was maximal at 30-60 minutes following exposure and lasted several hours. Protein kinase activity in the nuclear component of a cell homogenate increased over a period of up to 6 hours while activity in the cytosol fraction decreased in a near parallel fashion. The result suggests a redistribution of protein kinase activity from soluble to particulate fraction and perhaps translocation of protein kinase from the cell's cytosol to nucleus.

Since phosphorylation of nonhistone chromosomal protein has been implicated in the regulation of gene expression we decided to observe the pattern of phosphorylation of endogenous nuclear protein in neuroblastoma cells. Nuclei from control cells and cells treated for 15 minutes with 8 Br-cAMP were isolated and purified, then incubated under conditions to allow phosphorylation. We found that protein kinase activity in nuclei from cells treated with 8 Br-cAMP was increased 70-80% but showed no requirement for cyclic-AMP. To investigate the molecular nature of the protein phosphorylated, nuclei prepared from normal cells and cells treated with 8 Br-cAMP were extracted with sulfuric acid to remove histones. We found that about 80% of the total phosphorylation observed in nuclei was associated with the nonhistone protein.

We are now able to extend to the neuroblastoma cell some of our previous observations in adrenal medulla, namely 1) that second messengers can activate protein kinase as well as induce tyrosine hydroxylase and 2) that protein kinase may, in fact, be translocated to the nucleus where we have shown that acidic proteins are preferentially phosphorylated.

Publications:

Hollenbeck, R. A., Chuang, D. M. and Costa, E.: Characterization of protein kinases from adrenal medulla: A study of cytosol and nuclear enzymes. Neurochem. Res. 3: 49-67, 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 01492-06 SMRP

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Properties of monoamine oxidases

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	N. H. Neff	Chief	SMRP-B	NIMH
Other:	R. J. Wyatt	Chief	SMRC	NIMH

COOPERATING UNITS (if any)

Laboratory of Clinical Psychopharmacology

LAB/BRANCH
Laboratory of Preclinical Pharmacology

SECTION
Biochemical Pharmacology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, St. Elizabeths Hosp., Washington, D.C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to survey the types and activities of monoamine oxidase (E.C. 1.4.3.4) in autopsy material from individuals with various psychiatric disorders.

PHS-6040
(Rev. 10-76)

Project Description:

The monoamine oxidases (E.C. 1.4.3.4.:MAO) are enzymes which participate in the inactivation of biogenic amines. Drugs that inhibit MAO are used clinically as antidepressants and antihypertensives. The identification of Type A and B MAO (see M-SMR-P-12, 72-72) has prompted studies to define the role of these enzymes. Our objective was to continue our survey of the types and activities of MAO in autopsy material from individuals with various psychiatric disorders.

MAO activity was determined using serotonin (a substrate for Type A enzyme) or phenylethylamine (a substrate for Type B enzyme) as substrates.

Our study of human tissues for MAO activity in autopsy material is in progress.

Monoamine oxidase activity is low in blood platelets from schizophrenics and the low activity may have a heritable component. We are attempting to determine if enzyme activity in various tissues other than platelets is low in various psychiatric disorders.

MAO inhibitor drugs are used to treat hypertension and depression. Our studies may lead to a better understanding of depression and hypertension and how drugs such as MAO inhibitor might alleviate these disorders. They may also provide information on the etiology of some mental illnesses.

We will continue our investigation of human autopsy material for Type A and Type B monoamine oxidase activity.

Publications:

Fuentes, J. A. and Neff, N. H.: Inhibition by pargyline of cardiovascular amine oxidase activity. Biochem. Pharmacol. 26: 2107-2112, 1977.

Fuentes, J. A., Trepel, J. B. and Neff, N. H.: Monoamine oxidase activity in the cardiovascular system of young and aged rats. Exp. Gerontol. 12: 113-115, 1977.

Trepel, J. B., Wyatt, R. J. and Neff, N. H.: Differential distribution of Type A and B monoamine oxidase in neuronal tissues. Eur. J. Pharmacol. 43: 373-375, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do **NOT** use this space)

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 01497-05 SMRP

PERIOD COVERED

October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Using stable isotopes and mass fragmentography to study monoamine biosynthesis.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	C. L. Galli	Visiting Associate	SMRP NIMH
Other:	J. W. Commissiong	Visiting Associate	SMRP NIMH
	N. H. Neff	Chief	SMRP-B NIMH
	E. Costa	Chief	SMRP NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Preclinical Pharmacology

SECTION

Biochemical Pharmacology

INSTITUTE AND LOCATION

NIMH, ADAMHA, St. Elizabeths Hosp., Washington, D. C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to utilize the sensitivity and specificity of gas chromatography-mass spectrometry to study problems related to mental health. Our present investigations entail administering $^{18}O_2$ to rats and following its incorporation into tyrosine and then into dopamine and its metabolites.

PHS-6040
(Rev. 10-76)

Project Description: Z01 MH 01497-05 SMRP

This project has been terminated.

Publications:

Galli, C. L., Commissiong, J. W. and Neff, N. H.: Measuring the formation of the biogenic amines utilizing $^{18}O_2$. Biochem. Pharmacol. 26: 1271-1273, 1977.

Neff, N. H., Commissiong, J. W., Galli, C. L., Iuvone, P. M., Garrison-Gund, C. K. and Karoum, F.: Mass Spectrometry to measure regional aspects of dopamine metabolism. In Usdin, E., Weiner, N., and Youdim, M. H. B. (Eds.): Structure and Function of Monoamine Enzymes. New York, Marcel Dekker, 1977, pp. 781-797.

Galli, C. L., Commissiong, J. W., Karoum, F., Neff, N. H. and Costa, E.: Measurement of phenylalamine hydroxylating activity in vivo: $^{18}O_2$-tyrosine in plasma after exposing rats to $^{18}O_2$. Neurochem. Res. 2: 657-669, 1977.

Galli, C. L., Commissiong, J. W., Costa, E. and Neff, N. H.: Incorporation of $^{18}O_2$ into brain serotonin in vivo as a procedure for estimating turnover: a feasibility study in animals. Life Sciences. 22: 473-478, 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 01500-06 SMRP

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)
Indolealkylamines in the central nervous system

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. W. Commissiong	Visiting Associate	SMRP	NIMH
	L. M. Neckers	Staff Fellow	SMRC	NIMH
Other:	N. H. Neff	Chief	SMRP-B	NIMH
	E. Costa	Chief	SMRP	NIMH

COOPERATING UNITS (if any)
Laboratory of Clinical Psychopharmacology

LAB/BRANCH
Laboratory of Preclinical Pharmacology

SECTION
Biochemical Pharmacology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, St. Elizabeths Hosp., Washington, D.C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.4	0.4	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Our objective was to administer $^{18}O_2$ to rats and follow its incorporation into serotonin. In addition, we attempted to determine if serotonin synthesis in the striatum is modulated by a neuronal feedback loop.

Project Description:

Molecular oxygen is incorporated into the 5th position of the indole ring of serotonin during its synthesis in vivo, thus it should be possible to study the incorporation of $^{18}O_2$ into serotonin in vivo by using gas chromatography-mass spectrometry (GC-MS) methods.

The endogenous factors that modulate serotonin synthesis in striatum have not been adequately documented. We evaluated the possibility that serotonin is controlled by a negative neuronal feedback loop by destroying serotonin receptors by injecting kainic acid into the striatum. Kainic acid destroys the cell bodies of the neurons on which serotonergic neurons synapse.

Oxygen-18 was produced by the electrolysis of water containing ^{18}O (95 atom percent). The $^{18}O_2$ produced was administered to unrestrained rats in a closed homestatic chamber. Endogenous serotonin containing oxygen-18 was analyzed by GC-MS. Drugs were administered to evaluate whether ^{18}O incorporation into serotonin was by isotope exchange or enzymatic incorporation.

Kainic acid was stereotaxically injected into the striatum of rats and serotonin metabolism studied by high pressure liquid chromatography. Tryptophan hydroxylase activity was assayed by standard biochemical methods.

Serotonin containing ^{18}O instead of ^{16}O was found in brain after exposing rats to an atmosphere of $^{18}O_2$. The ^{18}O incorporation appeared linear with time and 22% of the serotonin in striatum contained ^{18}O after one hour of exposure. ^{18}O entered serotonin by enzymatic reaction rather than by isotope exchange as treatment with p-chlorophenylalanine, to block tryptophan hydroxylase, prevented incorporation, while more ^{18}O-serotonin than normal was found in brain after treatment with pargyline, to block monoamine oxidase. Our studies suggest that the incorporation of $^{18}O_2$ into brain serotonin might be a feasible approach for evaluating serotonin turnover in animals and with some modification might be applied to man.

Intrastriatal kainic acid caused a dose-dependent increase of ipsilateral striatal 5-hydroxyindoleacetic acid (5HIAA), the metabolite of serotonin, without affecting serotonin concentrations. The ipsilateral increase of 5HIAA was a reflection of increased serotonin in the injected striatum. Tryptophan hydroxylase was increased in the injected striatum as well. These studies provide experimental support for the existence of a negative feedback neuronal loop for regulating serotonin synthesis in striatum.

Abnormal serotonin metabolism has been implicated in several psychiatric and sleep disorders. These disorders might be related

to the failure of a negative neuronal feedback system for serotonin in brain. Presently there is no reliable method for studying serotonin metabolism in the brain of man. The possibility exists that by administering $^{18}O_2$ to man and analyzing the oxygen-18 incorporated into serotonin metabolites found in spinal fluid and plasma will be a useful method of evaluating serotonin formation in man.

In the future a detailed analysis of ^{18}O-serotonin formation in rat brain nuclei will be attempted. In addition, drugs that are known to alter serotonin metabolism will be evaluated in our test system.

Publications:

Galli, C. L., Commissiong, J. W., Costa, E. and Neff, N. H.: Incorporation of $^{18}O_2$ into brain serotonin in vivo as a procedure for estimating turnover: A feasibility study in animals. Life Sci. 22: 473-478, 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 01503-04 SMRP

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)
Pharmacological studies of acetylcholine turnover: control of septal-hippocampal pathway

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.	S. Robinson	Staff Fellow----	SMRP-NIMH
Other:	D. L. Cheney	Chief	SMRP-M-NIMH
	P. L. Wood	Guest Worker	SMRP-NIMH
	F. Moroni	Visiting Assoc.	SMRP-NIMH

COOPERATING UNITS (if any)
None

LAB/BRANCH
Laboratory of Preclinical Pharmacology

SECTION
Molecular Pharmacodynamics

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, St. Elizabeths Hosp., Washington, D. C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.7	1.4	0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Electrical stimulation of the septal-hippocampal pathway increases the turnover rate of acetylcholine in the hippocampus, whereas fimbrial lesions decreases the turnover rate. The indirectly acting dopaminergic and noradrenergic agonists, amphetamine and nomifensin increase the turnover rate of acetylcholine in the cortex and hippocampus. The excitatory effect of both drugs is blocked by intraseptal phenoxybenzamine. The directly-acting dopamine agonist, apomorphine, decreases the acetylcholine turnover rate in the hippocampus. The dopamine blocking agent haloperidol when injected directly into the septum increases the turnover rate of acetylcholine in the hippocampus. Injection of 6-hyroxydopamine into the septum or into Area A_{10} also increases the turnover rate of acetylcholine in the hippocampus. The directly-acting GABA agonist, muscimol, when given intraseptally decreases the turnover rate of acetylcholine in the hippocampus. Bicuculline is without effect when given directly into the septum.

PHS-6040
(Rev. 10-76)

Project Description

The objective of this study was to study the local neuronal circuitry that in septum controls the septal-hippocampus and septal-cortical cholinergic pathways by various neurotransmitters.

The methods for determining the concentration and turnover rate of acetylcholine were described in the Project MH 01509-08 SMRP - 1976. To study whether or not acetylcholine turnover rate was a reliable index of septal-hippocampus cholinergic neurons, the septal-hippocampal cholinergic pathway of the rat was either electrically stimulated or lesioned and the turnover rate of ACh was measured. An appropriate electrical stimulation of the septum selectively increased the turnover rate of acetylcholine in the hippocampus in non-anesthesized and in barbiturate-treated animals. The acetylcholine content of the hippocampus increases by approximately 30% one hour after the fimbria lesion, but decreased by about 80% nine days after the fimbria lesion. Acute fimbria lesion decreases the turnover rate of acetylcholine in the lesioned side by approximately 85% but the turnover rate of acetylcholine in the intact side and in the cortex was unchanged. The same is true in rats with chronic fimbria lesions. Thus, the hippocampal turnover rate of acetylcholine increases or decreases proportionally to the activity of the cholinergic neurons.

Administration of amphetamine (6.75 - 27 μmol/kg, 60 min i.p.) increased the turnover rate of acetylcholine in the cortex and hippocampus. Local administration of the irreversible alpha-adrenergic blocking agent, phenoxybenzamine, (15 nmole) into the septum blocks the increase in the acetylcholine turnover rate in the cortex and hippocampus, suggesting that cholinergic neurons projecting from the septum to the hippocampus or the cortex are excited by norepinephrine. Systemic administration of apomorphine (2.4 μmol/kg; 4.8 μmol/kg; 27 min, s.c.) caused a dose-dependent decrease in the turnover rate of acetylcholine in the hippocampus without changing that of cortex. This suggests that dopamine may have an inhibitory influence on the activity of cholinergic neurons projecting to the hippocampus, but not to those projecting to the cortex.

Injection of haloperidol (5 μg; 45 min), a dopamine blocking agent, directly administered into the septum increases the turnover rate of acetylcholine in the hippocampus but not in the cortex. This further suggests that there is a dopaminergic control of septal hippocampal but not of septal cortical cholinergic neurons. Furthermore, there is no change in the turnover rate of acetylcholine in striatum, suggesting that haloperidol has not diffused out of the septal area.

Destruction of dopaminergic nerve terminals in the septum by 6-hydroxydopamine (8 μg base; 11 days) also increases the turnover rate of acetylcholine in the hippocampus but not in the cortex or striatum. However, injection of 6-hydroxydopamine into the septum decreases noradrenergic as well as dopaminergic innervation of the area. Dopamine terminals in the lateral septum can be selectively destroyed by injection of 6-hydroxydopamine into area A_{10} in the ventromedial tegmentum. When 6-hydroxydopamine is injected into Area A_{10} the turnover rate of acetylcholine in the hippocampus,

is increased, but that of cortex is not.

Injection of the GABA agonist, muscimol (0.88 nmole) into the septum decreases the hippocampal turnover rate of acetylcholine. However, the GABA antagonist, bicuculline, has no effect. The lack of action by bicuculline may suggest that the GABA neurons do not exert a tonic influence on the firing of hippocampal cholinergic neurons. It has been proposed that alterations in the dynamics that regulate the transmitter function at the synaptic level may be involved in various forms of mental disease. Since neuronal activity is regulated by the rates of release and inactivation of transmitters, including acetylcholine, the study of the turnover rate of acetylcholine at various synaptic areas of rat brain associated with available anatomical and histological information is a tool to study the functional role of a cholinergic innervation in varrious brain structures of the limbic system. The frontal cortex and hippocampus are two areas of the brain which are closely associated with expressions of emotions, learning and cognitive processes. Cholinergic neurons are known to project to these areas and a study of the specific neurotransmitter control of the cholinergic neurons projecting to these areas may yield information that can be used in the design of drugs for the treatment of mental disease.

We shall continue to use local injections of the specific agonists and antagonists directly into specific nuclei of the rat brain to determine the transmitter control of the cholinergic dynamics.

Publications

Cheney, D. L. and Costa, E.: Biochemical pharmacology of the cholinergic neurons. In Lipton, M., Di Mascio, A. and Killam, K. (eds.); Psychopharmacology -- A Generation of Progress., New York, Raven Press, 1978, pp 283-291

Costa, E. and Cheney, D. L.: Regulation of cholinergic neurons by dopaminergic terminals. Influence of cataleptogenic and noncataleptogenic antipsychotics. In Garattini, S. (Ed.): Interations Among Putative Neurotransmitters in the Brain. New York, Raven Press, 1977, pp 23-28

Cheney, D. L. and Costa, E.: The transsynaptic regulation of acetylcholine metabolism in nuclei of rat brain. In Butcher, L. L. (Ed.): Cholinergic Monoaminergic Interactions in the Brain. New York, Academic Press, 1978, pp 229-245.

Robinson, S., Cheney, D. L., Moroni, F. and Costa, E.: Acetylcholine turnover in brain nuclei of rats injected with various antidepressants. In Proc. of a symposium on "Depressive Disorders",held at Rome, Italy, May 1978, in press

Moroni, F., Malthe-Sorenssen, D., Cheney, D. L. and Costa, E.: Modulation of acetylcholine turnover in the septal-hippocampal pathway by electrical stimulation and lesioning. Brain Res., in press

Robinson, S.E., Cheney, D. L. and Costa, E.: Effect of nomifensin and other antidepressant drugs on the acetylcholine turnover rate in various regions of the rat brain. Naunyn Schmiedeberg's Archives of Pharmacology, in press

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 01505-05 SMRP

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)
Anesthetics, REM sleep and acetylcholine turnover rate in rat brain

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	D. L. Cheney	Chief	SMRP-M	NIMH
Other:	W. Mendelson	Staff Psychiatrist	SMRC	NIMH

COOPERATING UNITS (if any)
Laboratory of Clinical Psychopharmacology and Dept. of Anesthesiology, Columbia University, New York, New York.

LAB/BRANCH
Laboratory of Preclinical Pharmacology

SECTION
Molecular Pharmacodynamics

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, St. Elizabeths Hosp., Washington, D.C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.4	0.1	0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Lidocaine, a local anesthetic, decreases the turnover rate of acetylcholine in cortex but not in striatum, hippocampus or diencephalon when administered in subconvulsive doses. Another local anesthetic, cocaine, increases the turnover rate of acetylcholine in cortex and diencephalon but not in striatum or hippocampus. These results suggest that the increase of acetylcholine turnover rate induced by cocaine may be unrelated to the local anesthetic effect and may be due to its CNS stimulant action.

Deprivation of REM sleep does not alter the turnover rate of acetylcholine in cortex, striatum, hippocampus or diencephalon.

PHS-6040
(Rev. 10-76)

Project Description:

Our objective was to study the mechanisms that regulate the function of cholinergic neurons in the central nervous system.

Acetylcholine and choline concentrations and acetylcholine turnover rate were measured mass spectrometrically in different regions of the rat brain after injecting two local anesthetics, lidocaine (100 mg/kg; s.c.) and cocaine (100 mg/kg; s.c.) and after REM sleep deprivation. Rats were deprived of REM sleep by sitting on an inverted flower pot (5 cm dia) for seven days. Several types of controls were used: (1) normal rats; (2) swim-stressed rats and (3) rats sitting on a larger inverted flower pot (10 cm dia).

Lidocaine produced a long lasting (90 min) sedation and cocaine caused a prolonged excitation (120 min). Under these behavioral conditions lidocaine reduced the turnover rate of acetylcholine in parietal cortex but had no effect in striatum, hippocampus or diencephalon. Cocaine, on the other hand, increased the turnover rate of parietal cortex and diencephalon but not in hippocampus or striatum. Cocaine produces a long-lasting CNS stimulating effect that is not shared by the other local anesthetics. The stimulation produced is comparable to that of amphetamine. In fact amphetamine (27 µmol/kg; i.p.; 60 min) also increases the turnover rate of acetylcholine in parietal cortex (Project No. Z01 MH 01503-03 SMRP, 1978). These results suggest that the increase of acetylcholine induced by cocaine may be unrelated to the local anesthetic effect and may be due to its CNS stimulating action.

Rats deprived of REM sleep did not have alterations in the turnover rate of acetylcholine in parietal cortex, striatum, hippocampus or diencephalon.

A clarification of the influences exerted by various anesthetic agents, as well as sleep, on cholinergic mechanisms may help in understanding the role that acetylcholine plays in consciousness.

The changes observed after lidocaine will be studied in greater detail using convulsive doses of the local anesthetic.

Publications:

Ngai, S. H., Cheney, D. L. and Finch, A.: Acetylcholine concentrations and turnover in rat brain structures during anaesthesia with halothane, enflurane and ketamine. Anesthesiology 48: 4-10, 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 01506-04 SMRP

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)
Narcotic analgesics and the regulation of dopaminergic pathways of rat brain

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.	L. Grandison	Staff Fellow	SMRP-NIMH
Other:	F. Moroni	Visiting Assoc.	SMRP-NIMH
	A. Guidotti	Chief	SMRP-N-NIMH

COOPERATING UNITS (if any)
None

LAB/BRANCH
Laboratory of Preclinical Pharmacology

SECTION
Neuroendocrinology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, St. Elizabeths Hosp., Washington, D. C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.6	0.6	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Analgesic doses of morphine increase striatal dopamine turnover rate but this increase does not appear related to either an increase in the firing rate of dopaminergic neurons or to the stimulation of postsynaptic dopaminergic receptors. Opiate receptors are located on dopamine (DA) nerve terminals and in unknown sites of substantia nigra and hypothalamus pituitary system when these three sites appear to be involved in the regulation of DA function. We have established that stimulation of opioid receptors located on the terminals of DA neurons in striatum increases HVA accumulation. In contrast, stimulation of opiate receptors in SN result in a blockade of the haloperidol-induced activation of the nigra-striatal DA neurons. The prolactin secretion was used as a tool to investigate the action of opiates on hypothalamic DA neurons. Our results suggest that the opiate receptors located in the hypothalamus toxically control the prolactin release by a mechanism which is not directly related to the function of the DA system.

Project description

Our objective was to study the action of narcotic analgesics administered acutely and chronically on the dopaminergic neuronal function. To establish whether narcotic analgesics interact with dopaminergic neurons at the pre- or post-synaptic receptor site we have studied changes in catecholamine turnover, of tyrosine hydroxylase (TH) activity in striatum, and in the prolactin release from the anterior pituitary.

1. Experiments in striatum:

Analgesic doses of morphine which increase the turnover rate of DA in rat striatum, produce an accumulation of HVA but fail to increase the striatal concentration of cyclic AMP or the affinity of TH for its pteridine cofactor. When morphine is added to striatal homogenates it changes neither the basal activity of adenylate cyclase nor the stimulation of activity induced by DA. Moreover, morphine fails to change the increase in striatal cAMP content caused by amphetamine, suggesting that it does not block the function of DA receptors. Recently, we observed that morphine injected systemically or intranigrally can block the haloperidol induced activation of striatal TH; however, morphine injected into the striatum fails to block the haloperidol induced activation of striatal TH. The activation of striatal TH induced by haloperidol is dependent on the blockade of postsynaptic DA receptors. Since morphine is devoid of a direct action on these receptors, it cannot counteract haloperidol at this site of action. It is, therefore, conceivable that opiate receptors on DA neurons of striatum and nigra directly or indirectly regulate the metabolism of DA in neurons. The next problem was to study whether opiate receptors in striatum and in nigra are located on DA neurons or on terminals of other neurons that release their transmitter in the vicinity of DA neurons.

The action of morphine on striatal HVA is apparently mediated by receptos located on striatal DA terminals. Morphine injected directly into the striatum increases HVA concentrations when all the morphine receptors with the exception of those associated with DA terminals, are destroyed. However, the blockade of haloperidol-induced activation of striatal TH by morphine is mediated by a nigral mechanism because intranigral injection of naltrexone reverses the action of morphine. Whether the opiate receptors present in SN are located in the DA cell bodies or in their dendrites or on the terminals of GABA or substance P neurons remains to be established.

2. Prolactin release from anterior pituitary

We used the hypothalamic pituitary axis as a model to elucidate the action of opiates on DA neuronal function. Prolactin (PRL) secretion from the pituitary is regulated predominantly by hypothalamic dopaminergic neurons that release DA which then inhibits secretion of PRL. Opiate receptor agonists stimulate PRL release while opiate receptor antagonists, such as naltrexone, block the opiate induced release as well as the basal PRL secretion. Thus, endogenous opiates participate in the physiological regulation of PRL release; perhaps, by interacting with DA neurons. The blockade

of DA receptors by haloperidol or the depletion of hypothalamic catecholamines by reserpine increase the blood levels of PRL because they increase the PRL release. Pargyline, a monoamine oxidase inhibitor, which increases hypothalamic DA, reversed the effects of reserpine on the PRL release. Naltrexone was ineffective in reducing the PRL release following reserpine or haloperidol, although it decreased the PRL release after estrogen treatmnt or stress. The increase in the PRL blood levels following endorphin failed to be blocked by pargyline, whereas naltrexone was effective. Thus, opiates and DA receptor activation operate differently in causing prolactin release.

The interrelations between opiate and DA receptor function are relevant to the mission of NIMH in many ways. Our results show that analgesia is associated with an increase of striatal dopamine turnover but this increase of dopamine metabolism does not appear to be related to either an increase in the firing rate of dopaminergic neurons or to the stimulation of postsynaptic dopaminergic receptors but to an action on DA terminals. In contrast, the opiate induced stimulation of PRL release is independent of DA neurons. Still to be determined is whether the blockade of haloperidol induced DA turnover by opiates occurs through a direct or indirect mechanism. Thus, a better understanding of the molecular and synaptic mechanisms involved may elucidate the basic mechanisms that control dopaminergic and enkephalinergic functions, two neuronal systems invovled in the etiology of mental illness.

Publications

Grandison, L. and Guidotti, A.: Regulation of prolactin release by endogenous opiates. Nature 270: 357-359, 1977

Guidotti, A. and Grandison, L.: Participation of hypothalamic endorphins in the control of prolactin release. In Costa, E. and Trabucchi, M (Eds.): Advances in Biochemical Psychopharmacology. New York, Raven Press, 1978 pp 191-198

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 01507-08 SMRP

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Possible neuronal alterations in muscular dystrophy

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	P. L. Wood	Guest Worker	SMRP NIMH
Other:	D. L. Cheney	Chief	SMRP-M NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Preclinical Pharmacology

SECTION
Molecular Pharmacodynamics

INSTITUTE AND LOCATION
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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.0	0.0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Genetically dystrophic mice (129 ReJ dy/dy) were found to possess a significantly lower brain acetylcholine content and turnover rate than littermate controls. Monoamine levels and turnover, however, were unaffected. These data suggest a central cholinergic defect which is presently being examined in various brain regions.

PHS-6040
(Rev. 10-76)

Project Description:

This project has been terminated.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 01508-08 SMRP

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)
Modulation by opiate receptors and antiagonists of the GABAergic and cholinergic function in various brain areas

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.	F. Moroni	Visiting Associate	SMRP-NIMH
Other:	D.L. Cheney	Chief	SMRP-M - NIMH

COOPERATING UNITS (if any)

None

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SECTION
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INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, St. Elizabeths Hospital, Washington, D. C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In rats, morphine (s.c.) and beta-endorphin (i.v.t.) administration decreases the turnover rate of GABA (TR_{GABA}) in the N. caudatus, but increases that of globus pallidus and substantia nigra. The effects of morphine on TR_{GABA} are reversed by naltrexone. The increase of TR_{GABA} in globus pallidus seems to play an important role in the morphine induced catalepsy. In fact, muscimol, a GABA receptor stimulating agent, once injected bilaterally into the globus pallidus mimics morphine catalepsy. Thus, the actions of morphine and beta-endorphin on the TR_{GABA} in caudate and globus pallidus may be due to an effect on opiate receptors localized in the head of the caudate nucleus. Since the TR_{GABA} in caudate decreases after opiate receptor administration and after surgical procedures which destroy the cortico-striatal afferents, it is possible that opioid neurons modulate the afferents to caudate and through these mechanisms modify the TR_{GABA}.

PHS-6040
(Rev. 10-76)

Project Description

Our objective was to monitor the function of the GABAergic neurons in various brain areas in order to ascertain whether or not stimulation of opiate receptors modify, in some way, the GABAergic function. In these studies we attempted to achieve a better understanding of the synaptic link between peptidergic neurons with opiate like activity and GABAergic neurons.

The procedures and the techniques for calculating TR_{GABA} have been described in Projects Z01 MH 01509-06 SMRP, 1975 and Z01 MH 01503-02 SMRP,1975. Catalepsy was measured according to Costall and Olley (Neuropharmacol. 10: 297, 1971). Polyethylene cannulae were implanted into the rat's ventricles according to Robinson et al., Physiol. Behav. 4: 123, 1969, and other cannulae were stereotaxically implanted into the N. caudatus, globus pallidus or substantia nigra using coordinates taken from Konig and Klippel Atlas (The Rat Brain, Williams and Wilkins, Baltimore, 1963). When morphine or beta-endorphin were injected intraventricularly the dose was contained in a volume of 10 μl of saline. In the animals with cannulae implanted in specific brain nuclei, the opioids were injected in volumes of 1-2 μl with the aid of a gear driven Hilton syringe. To lesion the cortico-striatal glutamate afferents, the frontal cortex was aspirated according to Kim et al., Brain Res, 1977. Other animals were hemitransected by means of a 3 mm long knife lowered with an angle of 60° at the level of the coronary suture (A.P. 8.2; Konig and Klippel). The knife was inserted next to the midline and subsequently moved outwards to achieve complete hemisection. Homovanillic acid was assayed by mass fragmentography using the procedures described by Oziedzic et al., J. Lab. Clin. Med. 82: 829, 1973. For the measurement of TR_{GABA}, the animals were killed by a beam of microwave radiation focussed on their skull. The brains were removed, mounted on a freezing microtome stage and sliced in 400 micron thick coronal sections. Various nuclei were punced out with stainless steel tubing (0.8 and 1.2 mm i.d.). The samples were homogenized, reacted and finally injected into a gas chromatograph-mass spectrometer for the determination of endogenous and $[^{13}C]_2$-glutamate and of endogenous and $[^{13}C]_2$ GABA. In the same punches HVA was also usually monitored.

Intraventricular injections of beta-endorphin induced analgesia and catalepsy as well as a dose dependent decrease of the TR_{GABA} in the caudate nucleus and a dose dependent increase of the TR_{GABA} in the globus pallidus and in substantia nigra. No changes in the GABA content were observed in the hippocampus or in the dentate gyrus. These effects were qualitatively similar to those induced by subcutaneous morphine administration. Naltrexone antagonized the effects of both morphine and beta endorphin. Since the TR_{GABA} may be a good biochemical index of the activity of the GABAergic neurons and since morphine and beta endorphin stimulate the enkephalinergic receptors present in the N. caudatus and in the globus pallidus, these experiments suggest that the release of enkephalin from enkephalinergic neurons inhibit the GABAergic function in the N. caudatus and increase that in the globus pallidus and substantia nigra.

In order to localize these interactions between the endogenous opiate receptors and GABA neurons, morphine and beta endorphin were injected locally

into the N. caudatus and the TR_{GABA} was measured in homolateral globus pallidus and substantia. In turn, these caudatal GABAergic neurons appear to interact with pallidal GABAergic neurons because the TR_{GABA} of pallidus increases when the output of GABAergic striatal neurons is decreased. These experiments demonstrated that morphine and beta endorphin directly interact with GABAergic neurons because they regulate the TR_{GABA} in N. caudatus. These conclusions were confirmed by injecting naltrexone into the head of the N. caudatus and injecting morphine subcutaneously. On the other hand, the increase of TR_{GABA} in substantia nigra elicited by parenterally administered morphine seems to be due to an activation or inhibition of local interneurons and not to an activation of long axons GABAergic strio-nigral neurons resulting from an inhibition of the GABA instrinsic interneurons.

The increase in the utilization of GABA elicited by the opioids in the globus pallidus may participate in determining catalepsy because muscimol a specific GABA receptor agonist, injected directly into the globus pallidus caused a dose related catalepsy. However, the increase in GABA utilization in substantia nigra is probably involved in other actions of morphine and beta-endorphin because muscimol, locally injected in this brain area failed to induce analygesia or to cause catalepsy.

Since the TR_{GABA} in the N. caudatus decreased also after ablation of the cortex or hemitransection, it is possible that the enkephalinergic neurons modulate the GABAergic striatal interneurons acting at the level of the cortico and nigra striatal pathways.

Due to the possible implications of the so called "strio-nigral loop" in severe neurological and psychiatric disorders, such as Parkinson's, Huntington's Chorea and some psychoses, biochemical studies evaluating the synaptic regulation of these pathways and the effect of drugs on these synaptic mechanisms may be of great practical interest. The study of the interactions between opioid peptides and putative neurotransmitters may clarify the brain modification leading to the development of tolerance and physical dependence and may help elucidate the pharmacology of morphine.

We plan to study the TR_{GABA} during the development of tolerance and physical dependence and to correlate the effects of morphine and opioid peptides on the TR_{GABA} with the behavioral effects of the two drugs. Furthermore, we plan to set up methods to investigate the effects of morphine and related peptides on other putative neurotransmitters such as glutamate, for example.

Publications

Moroni, F., Cheney, D. L. and Costa, E.: Beta-endorphin inhibits ACh turnover role in nuclei of rat brain. Nature 267: 267-268, 1977

Moroni, F., Cheney, D. L. and Costa, E.: Inhibition of acetylcholine turnover rate in rat hippocampus by intraseptal injections of beta endorphin and morphine. Naunyn Schmiedeberg's Archives Pharmacology 299: 149-153, 1977

Moroni, F., Cheney, D. L. and Costa, E.: The turnover rate of acetylcholine in brain nuclei of rats injected intraventricularly and intraseptally with alpha and beta endorphin. Neuropharmacology 17: 191-196, 1978

Costa, E., Fratta, W., Hong, J. S., Moroni, F. and Yang, Y.T.: Interactions between enkephalinergic and other neuronal systems. In: E. Costa and M. Trabucchi (eds.): Advances in Biochemical Psychopharmacology, Volume 18, New York, Raven Press, pp 217-266, 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01509-08 SMRP

PERIOD COVERED

October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Pharmacological studies of acetylcholine turnover peptides

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	P. L. Wood	Guest Scientist	SMRP	NIMH
Other:	D. L. Cheney	Section Chief	SMRP	NIMH
	D. Malthe-Sørenssen	Guest Scientist	SMRP	NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Preclinical Pharmacology

SECTION

Molecular Pharmacodynamics

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, St. Elizabeths Hospital, Washington, D. C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	1.1	0.4

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Somatostatin, α-MSH and ACTH1-24, injected intraventricularly augment the turnover rate of acetylcholine in the hippocampus. Surgical transection of the fimbria, cingulum or the perforant pathway does not block the intraventricular effect of these peptides. The results suggest that the increase in hippocampal turnover rate of acetylcholine may be caused by an interaction of the peptides with hippocampal receptors. Substance P and β-endorphin which decrease the turnover rate of acetylcholine when administered intraventricularly are still active when injected intraseptally suggesting that this action can be initiated by the activation of peptide receptors, located on the septum.

Project Description:

The present study was concerned with the role of peptide receptors in the regulation of the cholinergic septohippocampal pathway. Experimental details have been described in Project No. Z01 MH 01509-07 SMRP 1976 and 1977.

Somatostatin (SRIH), α-MSH and $ACTH_{1-24}$ were all found to augment the TR_{ACh} in the hippocampus. This action was specific for the hippocampus and was not observed in other brain areas. Local injections of the peptides into the septum and lesions of the fimbria/fornix demonstrated that this peptide action is independent from a direct or indirect activation of receptors impinging upon the cell bodies of septal cholinergic neurons. Acute section of the fimbria also failed to block the behavioral actions of these peptides injected intraventricularly while chronic sections did which cause a defiguration of acetylcholine neurons in hippocampus caused behavioral effects of dipeptides. These data verify the importance of the cholinergic terminals of the hippocampus in eliciting these behavioral repertoires.

Lesions of other neurons afferent to hippocampus (cingulum and perforant) pathway) also indicated that the above mentioned peptides did not act behaviorally via these pathways. Lesion of the arcuate nucleus, which contains the only known CNS α-MSH positive cells bodies, also did not modify the action of α-MSH on the hippocampal TR_{ACh}. These experiments suggest that when the examined peptides are injected intraventricularly they act directly at the level of the hippocampus and involve either peptidergic interneurons or afferent peptidergic fibers. Since supersensitivity did not develop after various fiber lesions the former possibility is more likely.

In contrast to the peptides discussed above, substance P and β-endorphin decrease the TR_{ACh} in the hippocampus (Project Z01 MH 01508-07, SMRP, 19). This action could be reproduced by intraseptal administration the receptors involved in this action are located within the septum. Further studies were undertaken to determine whether these inhibitory actions involved septal interneurons. Since intraseptal muscimol, a GABAergic agonist, decreases the TR_{ACh} in the hippocampus, the role of GABA interneurons was studied. The GABA receptor blocker bicuculline failed to block the decrease elicited by substance P on the hippocampal TR_{ACh}. These data indicate that GABA interneurons are not involved in the actions of substance P on the septal hippocampal cholinergic pathway. In contrast, bicuculline effectively blocks the β-endorphin induced decrease in the hippocampal TR_{ACh}. It, therefore, appears that GABA interneurons are probably intercalated between the cholinergic cell bodies and the β-endorphin afferents of the septum.

A variety of evidence suggests that pituitary releasing hormones exert nonpituitary brain effects which include behavioral and psychotic responses. To understand the mechanisms underlying these effects we condensed. The nonuniform distribution of these peptides in brain structures and studied their ability to specifically modulate the activity of cholinergic neurons in various brain nuclei we have assumed as a working hypothesis that the changes

they elicit in TR_{ACh} may be indicative of their involvement in modulating behavioral and physiological parameters through cholinergic mechanisms in that brain part.

These studies emphasize the important role peptides play in the regulation of the septo-hippocampal cholinergic pathway, which is an important regulator and synchronize neuronal activity of hippocampus and of its afferent neurons. It is known that limbic structure plays a key role in emotional behavior regulation. Further studies of the mechanism of these peptide actions are in progress as well as an analysis of the estimation of other potential peptide transmitters with hippocampal cholinergic neurons by increasing changes of TR_{ACh}.

Publications:

Cheney, D. L., Moroni, F., Malthe-Sørenssen, D. and Costa, E.: Endogenous modulators of acetylcholine turnover rate. In Jenden, D. J. (Ed.): Cholinergic Mechanisms and Psychopharmacology, New York, Plenum Press, 1978, pp 551-562

Wood, P. L., Malthe-Sorenssen, D., Cheney, D. L. and Costa, E.: Increase of hippocampal acetylcholine turnover rate and the stretching-yawning syndrome elicited by alpha-MSH and ACTH. Life. Sci. 22: 673-678, 1978

Malthe-Sorenssen, D., Wood, P. L., Cheney, D. L. and Costa, E.: Modulation of the turnover rate of acetylcholine by intraventricular injections of TRH, somatostatin, neurotensin and angiotensin II. J. Neurochemistry , in press

Malthe-Sorenssen, D., Cheney, D. L., and Costa, E.: Modulation of acetylcholine metabolism in the hippocampal cholinergic pathway by intraseptally injected substance P. J. Pharmacol. Exp. Ther., in press

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 01510-03 SMRP

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)
Interaction of GABA-ergic and cholinergic dynamics: Effect of Marijuana

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	A. Revuelta	Visiting Associate	SMRP	NIMH
Other:	D. L. Cheney	Chief	SMRP-M	NIMH
	F. Moroni	Visiting Associate	SMRP	NIMH
	P. L. Wood	Guest Worker	SMRP	NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Preclinical Pharmacology

SECTION
Molecular Pharmacodynamics

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, St. Elizabeths Hosp., Washington, D.C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Both Δ^9-THC and cannabinol (CBD) in high doses significantly increase the turnover rate of GABA in substantia nigra and reduce the turnover rate of acetylcholine in hippocampus and striatum. In hippocampus doses as low as 0.2 mg/kg Δ^9-THC but not CBD significantly reduce the turnover rate of acetylcholine. These results suggest that hippocampal cholinergic mechanisms may play a role in the psychotomimetic activity of Δ^9-THC.

PHS-6040
(Rev. 10-76)

Project Description:

This research has for its objectives the study of the effect of Δ^9-THC (the major psychoactive component of cannabis sativa) and CBD (an inactive component) on the TRACh and the TRGABA on various brain areas.

The techniques and methods used for the study of ACh were described in Project No. Z01 MH 01509-08 SMRP, 1976. The techniques and methods used for the study of GABA were described in the Project No. Z01 MH 01518-01 SMRP, 1976. Male Sprague Dawley rats were injected with ^{9}-THC (.2, .5, 0.5, and 10 mg/kg, i.v.) and CBD (10 mg/kg, i.v. and 20 mg/kg, i.v.). Δ^9-THC and CBD were suspended in 1% tween 80 10% propylene glycol and 89% 0.9% saline. Animals were sacrificed 30 min after i.v. injection.

We have observed that high doses of Δ^9-THC and CBD significantly increase the TRGABA in substantia nigra but do not affect the TRGABA in globus pallidus and striatum. Since both Δ^9-THC and CBD increase in the TRGABA in substantia nigra we can say that this effect is not related to the psychotomimetic action of Δ^9-THC.

Furthermore, we have measured the TRACh in various brain areas. High doses of THC and CBD decrease the TRACh in striatum which suggests that this effect may be nonspecific and unrelated to the psychoactive properties of cannabinoids. Low doses of Δ^9-THC decrease the TRACh in hippocampus which suggests that this molecule may act through a specific mechanism. CBD did not affect the TRACh in hippocampus or in other brain areas such as cortex or diencephalon. We observed that 5-10 mg/kg of Δ^9-THC produces catatonia and rigidity similar to the effect seen after 40 mg/kg of morphine. It has been suggested by other investigators that Δ^9-THC blocks the opiate withdrawal syndrome. Moreover it has been shown in our lab that morphine given intraseptally markedly reduced the TRACh in hippocampus and is reversed by naltrexone. We thought it interesting to find out whether or not Δ^9-THC is acting through the opiate receptors in the septum. We injected 5 mg/kg, i.p. naltrexone and 10 min later administered Δ^9-THC i.v. Naltrexone given in combination with Δ^9-THC did not reverse the inhibition of TRACh demonstrating that THC is not acting through opiate, the receptor in the septum.

Since small doses of Δ^9-THC shows significant decrease TRACh in hippocampus it became important to know whether Δ^9-THC exerts its effect directly on hippocampus or through the cholinergic pathway from the septum. Two hours after the fimbria was cut, THC was administered i.v. and the TRACh in hippocampus was measured. There was a decrease in the TRACh in the intact side. However Δ^9-THC on the cut fimbria side was not further reduced in the TRACh in hippocampus. This suggests that Δ^9-THC exerts its action on hippocampus through the cholinergic pathway from the

septum. It has also been found that substance P reduces the TRACh in hippocampus via an action on the system. The possibility exists that Δ^9-THC maybe exerting its effect by acting on substance P receptors. Therefore we have injected i.v. baclofen and Z-1150 (a substance P blocker related to muscimol) into the septum. Neither of the compounds by itself changes the TRACh in the hippocampus nor in the cortex and striatum.

Further studies will include experiments involving blockade of substance P receptors by baclofen or Z-1150 on the turnover rate of acetylcholine in substance P and Δ^9-THC treated rats.

From the biomedical point of view this investigation can help us to better understand the psychoactive effects of Δ^9-THC and other components of the widely used cannabis sativa. Furthermore this research can help to understand the characteristics of the receptors which are activated by Δ^9-THC.

Publications:

Revuelta, A. V., Moroni, F., Cheney, D. L. and Costa, E.: Effect of cannabinoids on the turnover rate of acetylcholine in rat hippocampus, cortex and striatum. Naunyn-Schmiedeberg's Arch. Pharmacol., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 01511-05 SMRP

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)
Neuronal monoamines: A study of their integration and regulation

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	A. Guidotti	Chief	SMRP-N	NIMH
Other:	F. Vargas	Guest Worker	SMRP	NIMH
	K. Kumakura	Visiting Fellow	SMRP	NIMH
	K. Gale	Staff Fellow	SMRP	NIMH

COOPERATING UNITS (if any)

None

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SECTION
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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.6	0.6	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The integrated function of dopaminergic, GABAergic and Substance P neurones was investigated in striatum and N. accumbens after short- and long-term treatment with antischizophrenic drugs. The data suggest that GABA and Substance P terminals in SN may be involved in mediating the effect of antischizophrenic drugs on nigro-striatal DA system. Long-term treatment with classical (cataleptogenic) antischizophrenic drugs produces tolerance to the activation of the DA striatal system. Tolerance is not observed after long-term treatment with atypical (non-cataleptogenic) antischizophrenic drugs. These data suggest that antischizophrenic drug-induced tolerance of the DA system in striatum is associated with DA receptor supersensitivity.

PHS-6040
(Rev. 10-76)

Project Description:

The heuristic value of the catecholamine (noradreneline and dopamine) hypothesis of affective disorders has been supported by several experiments during the past decade. In this process the body of research on catecholaminergic neuronal systems in patients with affective disorders and on various aspects of animal behavior has expanded.

However, it is today clear that catecholamines are not the only transmitter system that may be involved in the pathogenesis of affective disorders, in fact, not all aspects of the pathophysiology of various forms of affective disorders can be explained by simple alterations of catecholamine metabolism. Clearly, many other concomitant biochemical, physiological, and psychological factors must be involved.

During the last years, many studies have demonstrated the complex anatomical, physiological and biochemical interactions among monoaminergic and other neuronal systems. In this project we intend to study the consequences of short- and long-term treatment with antischizophrenic drugs on the dopaminergic and other synapses that interact with dopamine neurons.

Our studies have indicated that:

1) A single intraperitoneal injection of various antipsychotics with dopamine receptor blocking activity (methiothepin, haloperidol, pimozide, chlorpromazine, clozapine and thioridazine) decreases the apparent Km of tyrosine hydroxylase (TH) for the pteridine cofactor in striatum and nucleus accumbens. The doses of methiothepin, pimozide and haloperidol required to change the kinetic state of TH in striatum are lower than those required to produce similar changes in nucleus accumbens. In contrast, clozapine and thioridazine were more effective in nucleus accumbens than in striatum. Chlorpromazine was equally potent in both brain regions. Haloperidol increases the turnover rate of dopamine in striatum with doses that are relatively lower than those required for nucleus accumbens. In contrast, clozapine is more active in increasing turnover rate of dopamine in nucleus accumbens, while chlorpromazine was equally potent in both regions.

Thus, antipsychotics with a high incidence of extrapyramidal side effects (termed "classical") preferentially increase dopamine turnover in striatum. The antipsychotic with low incidence of extrapyramidal side effects (termed "atypical") preferentially influence the function of DA system in N. accumbens.

2) Discrete lesions of neuronal pathways descending from striatum to substantia nigra (SN) abolish the ability of anti-

psychotics to activate striatal TH. These lesions resulted in a 60-90% decrease of GABA and substance P in SN without changing DA content of nigra or striatum. Application of GABA-mimetic compounds such as muscimol or diazepam, either systemically or directly into SN, block the antipsychotic-induced activation of striatal TH. On the other hand, systemic administration of GABA antagonists such as picrotoxin or GABA synthesis blockers such as isoniazid potentiate the action of haloperidol. The data suggest that GABA terminals and possibly also substance P terminals in SN, mediate the effect of antipsychotic drugs on the activity of nigrostriatal DA neurons.

3) Long-term treatment with "classical" (cataleptogenic) and "atypical" (non-cataleptogenic) neuroleptics produces differential effects on the TH activity of locus coeruleus, C. striatum and N. accumbens. Rats treated for 10, 20 or 90 days with 0.5 mg/kg haloperidol have an increase in the activity of TH (Vmax) in locus coeruleus but not in striatum or nucleus accumbens.

Upon chronic haloperidol treatment and subsequent withdrawal for 3, 15 or 30 days, challenging doses of haloperidol or other "classical" neuroleptic drugs (pimozide, chlorpromazine, methiotepine) fail to increase the affinity of striatal TH for $DMPH_4$. Such tolerance to haloperidol was also present in N. accumbens however in this tissue the tolerance disappears 7 days after haloperidol withdrawal. After chronic haloperidol there is not cross-tolerance with the clozapine induced increase of striatal TH affinity for $DMPH_4$. Tolerance to long-term treatment with classical neuroleptics was not prevented by long-term treatment with Li^{++}. In rat treated chronically with clozapine, there was no tolerance to the effects on TH by challenging doses of clozapine or haloperidol.

These results indicate that long-term treatment with classical neuroleptics produces complex adaptive changes in the catecholamine system. The potential significance of these regulatory changes with regard to the therapeutic efficacy of the antipsychotic drugs is in favor of the hypothesis that mesolimbic DA is the most likely site when the control of affective disfunction by antipsychotics is operative.

In addition, the rapid and long lasting tolerance observed in striatum after classical neuroleptic administration relates to the process of supersensitivity of striatal DA receptors. This supersensitivity may be considered a biochemical determinant of the tardive dyskinesia which is observed in man chronically treated with classical neuroleptics, after discontinuation of the treatment or the reduction of the dosage.

Several reasons account for the differential development of tolerance and supersensitivity in striatum and N. accumbens, many others a different synaptic wiring could play a role. For instance, in N. accumbens but not in striatum, the GABAergic neurons are regulated by dopaminergic neurons. We, therefore, plan to determine more precisely the role of striato nigral pathway and striatal and accumbens interneuronal connections in the regulation of DA neuronal function. In particular, we want to study the respective influence of nigra and striatal substance P, GABA, and opiate peptides or opiate agonists and antagonists in the regulation of DA activity. In addition, we plan to study the role of cortico-striatal glutaminatergic projections. According to general clinical experience the full therapeutic response to a neuroleptic agent may require several weeks and may last for a considerable time after cessation of therapy. There are reasons to believe that the primary actions of neuroleptics, as observed in short-term experiments, is only the first response to induce a series of secondary effects, the nature of which has not yet been fully clarified. We thus, propose to extend our studies to the different neuronal system associated with the DA system and to long-term treatment with "classical" and "atypical" antipsychotics.

Publications:

Guidotti, A., Gale, K., Toffano, G. and Costa, E.: Regulatory role of GABA neurons on the activation of striatal tyrosine hydroxylase by haloperidol. In Weiner, N. and Usdin, E. (Eds.): Biochemistry and Function of Monoamine Enzymes. New York, Marcel Dekker, 1977, pp. 193-210.

Guidotti, A., Gale, K., Hong, J. and Toffano, G.: Models to study drug effects on the integrated function of GABAergic, peptidergic (substance P) and dopaminergic neurons. In Garattini, S., Pujol, J. F. and Samanin, R. (Eds.): Interactions Between Putative Neurotransmitters in Brain. New York, Raven Press, 1977, pp. 217-229.

Gale, K., Costa, E., Hong, J., Toffano, G. and Guidotti, A.: Evidence for a role of nigral GABA and substance P in the haloperidol induced activation of striatal tyrosine hydroxylase. J. Pharmacol. Exp. Ther., in press, 1978

Gale, K., Hong, J. and Guidotti, A.: Presence of substance P and GABA in separate striatonigral neurons. Brain Res. 136: 371-375, 1977.

Guidotti, A.: Tolerance to tyrosine hydroxylase activation in N. accumbens and C. striatum after repeated injections of "classical" and "atypical" neuroleptics". Life Sciences, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 01512-05 SMRP

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)
Transmitter interactions in the regulation of pituitary function

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	L. Grandison	Staff Fellow	SMRP	NIMH
Other:	M. Baraldi	Visiting Associate	SMRP	NIMH
	A. Guidotti	Chief	SMRP-N	NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Preclinical Pharmacology

SECTION
Neuroendocrinology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, St. Elizabeths Hosp., Washington, D.C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The role of GABA, catecholamine and endorphins in the response of hypothalamus to different psychoactive drugs was studied by monitoring eating behavior, pituitary hormone release and pituitary cAMP content. Eating behavior is under GABA, catecholamine and endorphin control. It appears that GABA neurons are distal to noradrenergic and endorphin containing neurons since blockade of GABA receptors inhibits eating induced by all three receptor agonist. Prolactin release is regulated by hypothalamic DA and endorphin but not by GABA receptors. The control of prolactin release by GABA occurs at the pituitary level. Pituitary cAMP is under independent DA, GABA and enkephalin control. Changes of cAMP content induced by these transmitter receptor agonist or antagonists are associated with release of ACTH.

PHS-6040
(Rev. 10-76)

Project Description:

Hypothalamic neuronal mechanisms transsynaptically regulate behavior and secretion of polypeptide releasing factors (RF's). The RF's, in turn, regulate pituitary function by stimulating adenylate cyclase in the anterior pituitary. In order to define the type of neurotransmitters involved in eating behavior and in the release of pituitary hormones, the effects of neuroactive drugs were monitored by the measurement of eating behavior, pituitary cAMP content and blood content of pituitary hormones.

Intraventricular or intrahypothalamic injection of muscimol, a potent GABA agonist stimulated voracious eating behavior for up to 30 minutes. The site of intrahypothalamic injection proved critical being effective only when located close to or within the ventromedial nucleus (VMN). This correlates with the satiety function proposed for the VMN and the site of action of other compounds that induce eating. The action of muscimol was blocked by bicuculline methyliodide, a specific GABA receptor antagonist. In addition bicuculline blocked eating induced by norepinephrine. However, phentolamine a blocker of noradrenergic receptors inhibited eating induced by norepinephrine but not muscimol. In addition beta-endorphin induced eating behavior and its action was blocked by naltrexone, an opiate receptor antagonist, and bicuculline but not phentolamine. Consequently regulation of eating behavior may involve noradrenergic, GABAergic and endorphin-containing neurons. It appears that GABA neurons are distal to noradrenergic and endorphin containing neurons since blockade of GABA receptors inhibits eating induced by all three agonists.

With regard to pituitary function it was found that depletion of hypothalamic biogenic amines induced by reserpine (10 mg/kg i.p.) resulted in a 4 fold increase in anterior pituitary cAMP content and in a 6-10 fold increase in serum prolactin (PRL) concentration. Administration of pargyline prior to reserpine blocked the increase in cAMP and prolactin. Depletion of hypothalamic GABA by subcutaneous (400 mg/kg s.c.) or intrahypothalamic (500 µg) injection of isoniazid was associated with a 5 to 10-fold increase in cAMP but no changes in serum PRL levels. Prior intrahypothalamic administration of muscimol prevented cAMP accumulation elicited by isoniazid. In contrast the cAMP accumulation following reserpine was not blocked by muscimol while the increase after isoniazid was unaltered by pargyline. In addition, intrahypothalamic injection of 1 µg beta endorphin stimulated PRL, growth hormone and cAMP accumulation while it decreases LH release. No changes were observed after intrahypothalamic injection of alpha endorphin, a non-opiate peptide. The actions of beta-endorphin were blocked by naltrexone. Muscimol blocked the increase in PRL induced by endorphin but only when given systemically rather than centrally thus suggesting effects of muscimol at the pituitary level. Pargyline was inactive in altering the

action of endorphin on the hypothalamic pituitary axis. These data suggest that catecholamines, GABA and endorphins are all involved in hypothalamic regulation of anterior pituitary functions. From these studies it appears that different neurotransmitter systems are involved in the release of specific anterior pituitary hormones. For example, PRL release is under hypothalamic DA and endorphins control but not under a direct hypothalamic GABA control. In contrast ACTH release is regulated by catecholaminergic and GABAergic neurons acting independently. Further efforts are now required to extend these studies to the numerous hypothalamic peptides neurotransmitter substances (neurotensin, somatostatin, substance P, bombesin) which have been shown to influence hormone release.

These studies are relevant to understanding total brain function because the hypothalamic neuronal peptides investigated here are now known to be widely distributed throughout the brain. The hormone release-neurotransmitter function relationship in the hypothalamus pituitary axis may be used as a tool to learn the action of different psychoactive drugs on neuropeptidergic transmission.

Publications:

Grandison, L. and Guidotti, A.: Stimulation of food intake by muscimol and β-endorphin. Neuropharmacology 16: 533-540, 1977.

Guidotti, A., Naik, S. R. and Kurosawa, A.: Possible role of gamma aminobutyric acid in the regulation of cAMP system in rat anterior pituitary. Psychoneuroendocrinology 2: 227-235, 1977.

Grandison, L. and Guidotti, A.: Regulation of prolactin release by endogenous opiates. Nature 270: 357-359, 1977.

Guidotti, A. and Grandison, L.: Participation of hypothalamic endorphins in the control of prolactin release. In Costa, E. and Trabucchi, M. (Eds.): Advances in Biochemical Psychopharmacology. New York, Raven Press, 1978, pp. 191-198.

Baraldi, M., Grandison, L. and Guidotti, A.: Brain distribution and metabolism of muscimol in rat. Neuropharmacology, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 01513-03 SMRP

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Synthesis of enzymes that regulate rate-limiting processes in control of the neuronal tissue.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	I. Hanbauer	Pharmacologist	IR HE -- H
Other:	E. Costa	Chief	SMRP -- NIMH

COOPERATING UNITS (if any)

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The goal of these studies was to elucidate the participation of hormones and the second messengers cyclic 3',5'-adenosine monophosphate (cAMP) or cyclic 3',5'-guanosine monophosphate (cGMP) in the regulation of tyrosine hydroxylase (TH) biosynthesis in tissues containing cells that synthesize catecholamines. Previous studies had shown that at least 3 types of receptors (β-adrenergic, nicotinic and corticosterone receptors) were involved in the induction of tyrosine hydroxylase in superior cervical ganglion. In the carotid body the induction of TH can involve corticosterone, muscarinic and oxygen receptors. The participation in the induction of a Ca^{2+} dependent activator protein is supported by the present experiments.

PHS-6040
(Rev. 10-76)

Project Description:

This project has been terminated.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 01514-06 SMRP

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Trans-synaptic control of protein synthesis

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	K. Kumakura	Visiting Fellow	SMRP	NIMH
Other:	A. Guidotti	Chief	SMRP-N	NIMH
	E. Costa	Chief	SMRP	NIMH

COOPERATING UNITS (if any)

None

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SECTION
Neuroendocrinology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, St. Elizabeths Hosp., Washington, D.C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.3	0.3	0

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In chromaffin cells of adrenal, the sequence of molecular events whereby trans-synaptic mechanisms regulate the genetic code expression includes an increase in the cAMP/cGMP concentration ratio, an activation of cAMP-dependent protein kinase (PK) in cytosol and the translocation of the low molecular weight catalytic subunit of this protein kinase from the cytosol to the subcellular particles. The PK of nuclei is not regulated by cAMP but it increases during the trans-synaptic induction of tyrosine-3-monooxygenase (TH) because the PK catalytic subunits translocate from cytosol to the nucleus. Thus, the activation and translocation of PK, triggered by the initial increase of cAMP due to the activation of PK acts as a long range messenger for the trans-synaptic expression of the genetic code in adrenal medulla. Using primary culture of adrenal chromaffin cells, we are now trying to understand the molecular mechanisms that in chromaffin cells regulate transcription of genes after translocation of PK from cytosol to the nucleus.

Project Description:

Our objective was to study in adrenal medulla the synthesis regulation of specific proteins by trans-synaptic mechanisms or by the injections of cholinergic receptor agonists.

An increase of cAMP/cGMP concentration ratio is a stimulus-coupled biochemical event occurring in adrenal medulla of rats during direct or transsynaptic stimulation of medullary nicotinic receptors. When this second messenger response lasts 50 minutes or more it elicits a delayed increase in protein synthesis including that of TH molecules.

We studied with great detail the cascade of macromolecular events elicited trans-synaptically in the enterochromaffin cells of rat adrenal medulla because we thought it could be a model of the macromolecular mechanisms mediating the changes in nuclear metabolism elicited by the activation of membrane bound receptors for transmitters of nerve impulses. In chromaffin cells for the regulation of nuclear metabolism it appears of critical importance that the increase in the cAMP content, elicited by the sustained activation of nicotinic receptors, lasts at least 1 hr; as an interpretation of this stringent requirement we have proposed that catalytic subunits of the cAMP-dependent protein kinase must remain dissociated for a sufficient time interval from the regulatory subunits in order to allow for their transfer in appropriate or sufficient numbers from cytosol into the nucleus. In support for this proposal we have found that a dissociation of catalytic subunits of PK lasting 2 to 3 hrs appears to be an obligatory event that mediates the long lasting increase in TH synthesis which is associated with the transsynaptic induction of TH. During this time we have estimated that 30 to 40% of the catalytic subunits of cAMP dependent PK translocate from the cytosol to the subcellular particles. This translocation of PK molecules persists longer than 16 hrs. This persistance appears to depend on the integrity of the cholinergic innervation of the chromaffin cells because in denervated adrenal medulla, stimuli, such as treatment with aminophylline which increases cAMP for longer than 1 hr and activates the cAMP-dependent PK of cytosol for the required time period no longer produces the persisting translocation. Moreover if denervation is produced after activating cAMP dependent PK translocation of PK terminates shortly after denervation. Whenever translocation lasts less than 10 hrs, TH fails to be induced.

These and other results suggest that in chromaffin cells the activation of cAMP-dependent PK and its translocation from cytosol to subcellular structures are obligatory intermediary processes for transsynaptic induction of TH which are triggered by the early increase of cAMP due to the activation of membrane bound acetylcholine receptors.

In future studies we plan to use primary culture of bovine adrenal medulla to study whether these molecular events including the increase in nuclear phosphorylation are necessary to regulate the expression of the genetic code during the induction of TH.

Initial experiments indicate that isolated bovine adrenal chromaffin cells maintained in tissue culture medium synthesize and release catecholamines

in a fashion similar to that expected for normal adrenal cells. In addition, 8-Bromo-cyclic AMP and nicotine can induce tyrosine hydroxylase activity. The induction of tyrosine hydroxylase by these agents is blocked by cycloheximide and by concentrations of calchicine (10^{-8} M) which do not inhibit protein synthsis but block microtubulin polymerization. These results suggest that the primary culture of bovine adrenal medulla can be a useful model for future studies on the translocation using macromolecular injection of catalytic subunits of PK and the study of the transcription of genes including those involved in TH induction.

Since in the adrenal medulla, the availability of catecholamines and the adrenergic function are regulated by tyrosine hydroxylase and since adrenergic mechanisms have been implicated in the etiology of affective disorders, an understanding of the molecular nature of the regulation of biosynthesis of catecholamines may contribute to a better understanding of the synaptic defects that may be operative in the etiology of mental diseases and of the basic mechanisms that may be involved in memory and learning.

Publications

Guidotti, A. and Costa, E.: The induction of tyrosine 3-monooxygenase in rat adrenal medulla: a model for the transsynaptic regulation of gene expression. In Harber, B. and Aprison, M. H. (Eds.): Neuropharmacology And Behavior. New York, Plenum Press, 1978, pp 103-125

Costa, E., Chuang, D. M. and Guidotti, A.: Adrenal medulla: a model to study the transsynaptic regulation of gene expression. In Weiner, N. and Usdin, E. (Eds.): Proceedings of the Symposium on Biochemistry and Function of Monoamine Enzymes, New York, Marcel Dekker, 1977, pp 279-310

Costa, E. and Guidotti, A.: Molecular mechanisms mediating the transsynaptic regulation of gene expression in adrenal medulla. In Lipton, M. A., DiMascio, A. and Killam, K. F. (Eds.): Psychopharmacology: A Generation of Progress. New York, Raven Press, 1978, pp 253-246

Guidotti, A. and Costa, E.: Commentary on Transsynaptic regulation of tyrosine 3-monooxygenase biosynthesis in rat adrenal medulla. Biochem. Pharmacol. 26: 817-823, 1977

Costa, E., Chuang, D. M., Guidotti, A. and Hollenbeck, R.: Control of nuclear function in chromaffin cells by persistent activation of nicotinic receptors. In Jenden, D. J. (Ed.): Cholinergic Mechanisms and Psychopharmacology. New York, Plenum Press, 1977, pp 267-284

Guidotti, A., Chuang, D. M., Hollenbeck, R. and Costa, E.: Nuclear translocation of catalytic subunits of cytosol cAMP dependent rpotein kinase in the trans-synaptic induction of medullary tyrosine hydroxylase. In George, W. J., and Ignarro, L. J. (Eds.): Advances in Cyclic Nucleotide Research. New York, Raven Press, 1978, pp. 185-197.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01515-05 SMRP

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Short and long term biochemical changes in cerebellum after treatment with psychoactive drugs.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. Baraldi	Visiting Associate	SMRP NIMH
	E. Bandle	Visiting Associate	SMRP NIMH
Other:	A. Guidotti	Chief	SMRP-N-NIMH

COOPERATING UNITS (if any)

None

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SECTION
Neuroendocrinology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, St. Elizabeths Hosp., Washington, D. C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.6	0.6	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Biochemically, the cerebellum is characterized by a large concentration of GABA, cGMP, cGMP-dependent protein kinase and a heat-stable protein which inhibits the cGMP-dependent protein kinase. Drug-induced changes in GABA receptor function can be easily monitored by measuring changes in these biochemical parameters. It is suggested that diazepam and muscimol (an indirect and a direct GABA receptor agonist), modulate cerebellar phosphorylation by altering the relationship between cGMP-dependent protein kinase and the content of the cerebellar inhibitor of protein kinase. They could act on the content of the protein kinase inhibitor by decreasing the cerebellar cGMP content without modifying that of cAMP.

Project Description:

The cerebellum is the brain region where the synaptic interactions among various neurons has been determined with greater detail and precision. The project is designed to relate changes of cAMP, cGMP content and protein kinase activities with the release of transmitters in the cerebellar cortex and deep cerebellar nuclei. Such studies are possible in cerebellum because:

1. The climbing fibers and the mossy fibers are the two major excitatory inputs to the Purkinje cells.

2. The function of Purkinje cells is regulated by inhibitory (GABAergic) interneurons.

3. The Purkinje cells are the sole output from the cerebellum and release GABA on the deep cerebellar neurons.

Previous reports have shown that pharmacological manipulations of climbing or mossy fibers not only change the firing pattern of Purkinje cells but also increase the cGMP content of the cerebellar cortex. Similarly, manipulation of the cerebellar GABAergic function changes the firing rate of the Purkinje cells and the cerebellar cGMP content. When the concentration of cerebellar GABA is increased or GABA receptors are stimulated, the firing rate of Purkinje cells and the cGMP content of cerebellar cortex are reduced. Conversely, when GABA receptors are blocked, the firing rate of Purkinje cells and the cerebellar cGMP content are increased.

Lesion of the climbing fiber system with 3-acetylpyridine blocks the increase of cGMP produced by harmaline (a selective activator of the olivocerebellar pathway) but leaves unabated the increase of cGMP produced by glutamic acid (an excitatory putative amino acid transmitter) or by isoniazid (a drug that decreases GABA levels). Apomorphine increases cerebellar cGMP, and haloperidol or morphine, decreases cerebellar cGMP when injected in striatum but not in cerebellum, whereas, diazepam decreases cerebellar cGMP when injected in cerebellum but not in striatum. These results suggest that two excitatory transmitters released by the parallel or climbing fibers and GABA released by cerebellar interneurons are independently involved in changing cGMP content on the molecular layer of cerebellum.

As we have previously reported (Smigielski, A., Guidotti, A., Costa, E. J. Biol. Chem. 252: 3848-3853, 1977) the rat cerebellum contains a significant amount of cGMP-dependent protein kinase and a large concentration of protein kinase inhibitors. These inhibitors are thermostable proteins which can be separated by gel chromaotgraphy into 2 molecular forms: the Type I and Type II inhibitor of protein kinase. The Type I inhibitor blocks the rat cerebellar cGMP-dependent protein kinase activity while the Type II inhibitor blocks the rat cerebellar cGMP-dependent protein kinase and the cyclic nucleotide independent protein kinase. The activity of the Type II inhibitor increased or decreased in opposite direction to changes of cerebellar cGMP content generated by injection of harmaline or diazepam. No changes of Type I

inhibition were observed under these conditions.

The drug-induced shift of Type II inhibitor of protein kinase was not mediated by changes in protein synthesis because it persisted after pretreatment with cycloheximide. These results are compatible with the hypothesis that cGMP modulates phosphorylation in cerebellum by changing the relationship between cGMP-dependent protein kinase and Type II inhibitor content. Experiments were then directed to elucidate the possible message mediated by the increase of cGMP in the cerebellum. To achieve this goal our attention has been focused on cGMP dependent protein kinase and on the thermostable protein inhibitors of protein kinase dependent phosphorylation. We have developed a simple method to assay cGMP-dependent protein kinase which allows the simultaneous processing of a large number of samples. Using this method we have established that cerebellar cGMP dependent protein kinase is very low at birth and increases with age in coincidence with several ontogentic events in cerebellum. One of the events that more closely relate with the increase of cGMP dependent protein kinase is the formation of a permanent synaptic contact between climbing fibers and Purkinje cells. In addition using mutant mice (nervous, weaver, staggerer) and rats treated with 3-acetylpryidine we further established a fundamental role of intact climbing fiber-dendritic synaptic contacts for the maintanence of normal cGMP-dependent protein kinase activity in cerebellum.

Considering the important role of the cerebellum in the regulation of the sensory motor cortex, function, and the large abundance of inhibitory (GABA, taurine) or excitatory transmitters (aspartic and glutamic acid) it is conceivable that studies on the modification of cerebellar cGMP may become an important tool to study the action of anti-psythotic tranquilizers and antidepressant drugs.

Our proposed course is to define the molecular nature of the messages mediated by the increase of cGMP. We have now established that rat cerebellum contains (1) a large number of cGMP dependent protein kinase and (2) high concentrations of a thermostable small molecular protein which inhibits cAMP dependent and cGMP dependent protein kinase.

We intend to study the transmitter-mediated changes of cerebellar cGMP-depdendent protein kinase and the interaction of the cGMP protein kinase with the inhibitor protein.

Publications:

Biggio, G., Costa, E., Guidotti, A.: Regulation of 3/5/-cyclic guanosine monophosphate content in deep cerebellar nuclei. Neuroscience 2: 49-52, 1977.

Guidotti, A., Biggio, G. and Costa, E.: Action of diazepam, haloperidol, morphine and muscimol on the cGMP content of cerebellum. In Deniker, P., Raduco Thomas, C. and Villeneuve, A. (Eds.): Neuropsychopharmacologicum.

Oxford, England, Pergamon Press, 1978, in press.

Biggio, G., Brodie, B. B., Costa, E., Guidotti, A.: Mechanisms by which diazepam, muscimol and other drugs change the content of cGMP in cerebellar cortex. Proc. Natl. Acad. Sci. U.S.A. 74: 3592-2596, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01516-05 SMRP

PERIOD COVERED

October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Biochemical pharmacology of minor tranquilizers

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	G. Toffano	Guest Worker	SMRP	NIMH
Other:	M. Baraldi	Visiting Associate	SMRP	NIMH
	A. Guidotti	Chief	SMRP-N	NIMH
	D. Konkel	Chemist	SMRP	NIMH
	E. Costa	Chief	SMRP	NIMH

COOPERATING UNITS (if any)

Department of Pharmacology, George Washington University

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SECTION

Neuroendocrinology

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NIMH, ADAMHA, NIH, St. Elizabeths Hospital, Washington, D. C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The molecular mechanism whereby GABA system is modified by benzodiazepines (BDZs) was studied in vivo and in vitro. In vivo BDZ like the GABA mimetic, muscimol antagonize the isoniazid-induced convulsions, and the isoniazid-induced increase of cerebellar cGMP and pituitary cAMP content. In vitro BDZs facilitate binding of GABA to its receptor sites by competing with the endogenous membrane protein which acts as an inhibitor of high affinity Na^+-independent GABA binding to synaptic membrane preparations. The same endogenous inhibitor of GABA binding competes with BDZs for their specific receptor sites. This increase in the binding affinity of GABA for its postsynaptic site may represent the molecular mechanism by which BDZs facilitate GABA transmission in vivo. These observations may help in elucidating details of the supramolecular nature of postsynaptic GABA and BDZs receptors and also may offer a simple means for studies of the biochemical defects in anxiety states.

PHS-6040
(Rev. 10-76)

Project Description:

Recent evidence from this laboratory has suggested that benzodiazepines, which are the most widely prescribed anti-anxiety drugs, may exert a primary action on GABA mechanisms. The present project intends to further elucidate the molecular mechanisms of action of benzodiazepines.

BDZs like muscimol (a direct GABA receptor agonist) and intraventricular nipecotic acid (a blocker of GABA uptake) delayed the onset of convulsions induced by isoniazid in doses that did not protect the animals from strychnine convulsions. Moreover, diazepam and muscimol had a similar rank order in the potency to prevent the increase of cerebellar cGMP or pituitary cAMP induced by isoniazed, picrotoxin, harmaline and reserpine. In the nigrostriatal system BDZs, like other GABA receptor agonists, blocked the activation of DA neurons induced by antipsychotics. These data concur with the hypothesis that the pharmacological action of BDZs may be mediated by an effect on GABA neurons.

To examine at the molecular level whether BDZs changed the kinetic of ^{3}H-GABA binding to its postsynaptic receptor we measured whether BDZs changed GABA binding to its postsynaptic receptor, we measure whether BDZs changed GABA binding to fresh or frozen (triton-treated) crude synaptic membrane preparations from rat cerebral cortex, using a Na^+ free medium. We found that BDZs uncovered high affinity binding sites in fresh membrane preparations but failed to modify GABA binding in frozen, thawed, triton treated and repeatedly washed membranes, which have these high affinity binding sites already exposed.

Freshly prepared synaptic membranes contain an acidic protein which inhibits the Na^+-independent high affinity binding of ^{3}H-GABA. This inhibitor is thermostable, has a MW of about 15,000 and has been purified 500-fold. When the membranes are frozen, thawed, repeatedly washed and then treated with Triton X-100 the inhibitor dissociates from the membranes. Kinetic analysis suggests that the inhibitor acts as an allosteric modulator for the Na^+-independent high affinity GABA binding. Diazepam (5×10^{-7} M) prevents the inhibition by this protein of ^{3}H-GABA binding to frozen and detergent-treated membranes. When diazepam is added to freshly prepared synaptic membranes of rat cerebral cortex, it unravels a high affinity component of ^{3}H-GABA binding (KD approx. 30 nM). Similar results were obtained using clonazepam, flunitrazepam, nitrazepam and (+)-RO-11-3128 (an active BDZ) but not (-)-RO-11-3624 (an inactive isomer).

These results suggest that BDZs compete with the endogenous inhibitor for the Na^+-independent high affinity binding of GABA. It has been reported that benzodiazepines work on specific "anxiety" receptors. In recent experiments we have observed that the endogenous inhibitor of GABA binding also inhibits ^{3}H-diazepam binding. The interaction between the endogenous protein and ^{3}H-diazepam is a competitive type thus suggesting that the endogenous protein inhibitor may represent the natural ligand for BDZs receptor sites.

In conclusion, our data suggested that BDZ receptors are functionally associated with GABA receptors. Thus, the pharmacological and therapeutic action of BDZs may be mediated by an action on GABAergic neurons.

The importance of these observations may go beyond the immediate explanation of the mode of action of BDZs and may represent the beginning of a better understanding of the role of GABAergic neurons in anxiety and in other mental disorders. In addition, these observations may help in elucidating details of the supramolecular nature of postsynaptic GABA and BDZ receptors and also may offer a simple means for studying the GABAergic pathway involved in anxiety.

Publications:

Guidotti, A., Naik, J. R. and Kurosawa, A.: Possible role of gamma aminobutyric acid (GABA) in the regulation of cAMP system in rat anterior pituitary. Psychoneuroendocrinology 2: 227-235, 1977.

Guidotti, A.: Methods to evaluate in vivo the activity of GABA receptor agonists. In Deniker, P., Raduco-Thomas, C. and Villeneuve, A. (Eds.): Proceedings of the 10th Congress of Collegium Internationale Neuropsychopharmacologium. Oxford, England, Pergamon Press, in press.

Guidotti, A.: Synaptic mechanisms in the action of benzodiazepines. In Lipton, M. A., DiMascio, A. and Killam, K. F. (Eds.): Psychopharmacology: A Generation of Progress. New York, Raven Press, 1978, pp. 1349-1358.

Biggio, G., Costa, E., Brodie, B. and Guidotti, A.: Mechanisms by which diazepam, muscimol and other drugs change the content of cGMP in cerebellar cortex. Proc. Nat. Acad. Sci. USA 74: 3592-3596, 1977.

Toffano, G., Guidotti, A. and Costa, E.: Purification of an endogenous protein inhibitor of the high affinity binding of GABA to synaptic membranes of rat brain. Proc. Nat. Acad. Sci. USA, in press.

Guidotti, A., Toffano, G., Grandison, L. and Costa, E.: Second messenger responses and regulation of high affinity receptor binding to study pharmacological modifications of GABAergic transmission. In Fonnum, E. (Ed.): Amino Acids as Chemical Transmitters. New York, Plenum Publ., in press, 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01517-03 SMRP

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Development of a high power microwave oven

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	A. Guidotti	Chief	SMRP-N	NIMH
Other:	D. L. Cheney	Chief	SMRP-M	NIMH
	J. Meek	Pharmacologist	SMRP	NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Preclinical Pharmacology

SECTION
Neuroendocrinology and Molecular Pharmacodynamics

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, St. Elizabeths Hospital, Washington, D. C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Focussed microwave irradiation equipment is described which while not impairing microdissection of rat brain nuclei, spinal cord and other tissues allows the assay of the concentrations of chemicals which undergo rapid metabolism during postmortem processes.

We are now developing new microwave devices to inactivate enzymes in brain and other tissues of large animals and in free moving animals.

This equipment will offer a unique possibility of measuring the effect of drugs on the steady state and turnover rate of neurotransmitters of metabolites during various stages of sleep.

PHS-6040
(Rev. 10-76)

Project Description:

Transmitter and second messenger content of brain structures undergo rapid postmortem fluctuations.To measure concentrations and turnover rates of these molecules, it is necessary to instantaneously inactivate the enzymes that synthesize and metabolize these molecules to minimize postmortem distraction. Microwave radiation has been proposed as a method for killing animals in order to preserve brain structure (Stavinoha et al., Pharmacologist 12: 257, 1970).

A metal wave guide connects the microwave generator (2 KW, 2.45 GHz) to a power window opening. A rat or mouse is placed in an animal holder made of plastic which is mounted under the power window opening with the animals's head exposed to the microwave beam. An electronic system precisely controls the duration of radiation (0.5 sec for mice and 2 sec for rats).

To ascertain that this technique is suitable for measuring concentrations of various constituents in brain of rats, microwave irradiation was compared with the rapid freeze blowing technique (J. Neurochem. 20: 183-188, 1973). With this procedure, high pressure liquid N_2 is used to blow the brain out of the cranial cavity and freeze it within 0.5 sec. With the procedure that uses microwave radiation, however, it is possible to dissect brain nuclei; dissection is impossible following freeze blowing procedures.

The concentration of a number of endogenous substances in the brain of rats killed by microwave radiation was comparable to that found after freeze-blowing, suggesting that microwave killing was not facilitating the accumulation of brain substrate after death.

Currently, we are attempting to increase the value of microwave radiation in studying putative transmitters in spinal cord and brain of rats by increasing the intensity of the beam. The new microwave source which we are describing will allow a rapid (2-3 sec.) inactivation of the enzyme controlling cAMP and cGMP metabolism in spinal cord and in organs like kidney, spleen, heart, etc., without handling the animals. Moreover, we plan also to develop this high power microwave generator that will allow us to focus the microwave on the skull of an unrestrained animal. This technique should allow important progress in the studies of different neurotransmitters and metabolites in spinal cord or brain during various stages of sleep.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01518-02 SMRP

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

The study of mammalian GABA-ergic mechanisms with gas chromatography-mass spectrometry and stable isotopes

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	E. Peralta	Guest Worker	SMRP	NIMH
Other:	F. Moroni	Visiting Associate	SMRP	NIMH
	E. Costa	Chief	SMRP	NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Preclinical Pharmacology

SECTION
Molecular Neurobiology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, St. Elizabeths Hosp., Washington, D.C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.6	0.6	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A technique for the estimation of the turnover rate of GABA (TRGABA) using gas chromatography and mass fragmentography has been applied to the study of the interrelationship between dopamine and GABA in the striatum and N. [illegible]. The effect of electroconvulsive shock (ECS) on TRGABA in various regions of the brain has also been studied. Our results show that electrical stimulation of the head of N. caudatus produces a significant increase in the TRGABA in the substantia nigra. However, stimulation of the substantia nigra does not have a clear effect on the TRGABA of homolateral N. caudatus suggesting an indirect relationship between dopaminergic input to these nuclei and local GABA neuronal regulation. Contralateral caudate is however affected during an indirect connection probably via cortex. In rats, repeated ECS increases GABA concentrations in the N. caudatus and N. accumbens and decrease the TRGABA by 40% and 50%, respectively, in these regions. Subconvulsive shocks have no effect. No consistent changes have been seen in substantia nigra.

Project Description:

Our objective has been to study the various populations of GABAergic post-synaptic cells which are regulated transsynaptically by the dopaminergic input to the N. caudatus and N. accumbens. With this study we intend to clarify whether or not previously reported increases in the turnover rate of GABA (TRGABA) in various telencephalic nuclei, elicited by antipsychotic drugs, can be attributed to the dopamine-receptor blocking effects of these agents.

Similarly, we have attempted to contribute to a possible implication of GABAergic mechanisms in the modifications elicited in rats by repeated electro-convulsive shocks (ECS).

We have used a sensitive gas chromatographic-mass fragmentographic technique developed in our laboratory to estimate GABA and glutamate and to measure their metabolic rate in brain nuclei. We have coupled this method with the infusion of ^{13}C uniformly labelled glucose to estimate the synthesis rate of GABA (see Project No. Z01 MH 01518-01 SMRP, 1976). Our studies have been carried out in three discrete nuclei of the rat brain; N. caudatus, N. accumbens and substantia nigra. While we are also interested in studying the TRGABA in hippocampus and cerebellum, this has not been possible so far with the technique available, due to the existence in these latter areas of a big, glutamate pool with fast turnover which renders unapplicable the assumption that determine the theortical model of precursor-product relationship used for our calculations of TRGABA. Electrical stimulation of the head of N. caudatus produces a significant increase in the TRGABA in the homolateral substantia nigra as compared to control side. Conversely, surgical deafferentiation of the GABAergic strio-nigral pathway leads to a decrease in nigral TRGABA one week after surgery. These results indicate that the measurement of TRGABA is a good biochemical index of the functional activity of a population of GABA-ergic terminals.

Electrical stimulation of the substantia nigra or A10 area produces an increase in the homovanillic acid (HVA) content of the homolateral caudate nucleus or nucleus accumbens, respectively. However, no consistent changes in TRGABA could be detected in connection with this enhanced dopaminergic activity. Associated with a 100% increase in the HVA content of the caudate nucleus ipsilateral to the stimulation, there was a significant decrease in TRGABA in the contralateral caudate. Presently we are investigating whether this is a specific effect mediated through the increase in the dopaminergic activity ipsilateral to the nerve stimulation.

Administration of ECS to rats results in their displaying enhanced behavioral responses which are believed to be monoamine-mediated. Since little is known on the mode of action of ECS in the therapy of depression, we have investigated whether a reduction in the modulatory influence of neurotransmitters due to an increase in GABAergic activity was operative. In our hands, repeated ECS (ten daily sessions) increased GABA concentrations in N. caudatus and N. accumbens and decreased the TRGABA by 40% and 50% respectively in these regions. Subconvulsive shocks (10 daily sessions) or a single convulsive shock had no effect. No consistent changes were seen in the substantia nigra with any of

the aforementioned treatment patterns. These results suggest that repeated ECS result in a decreased inhibition by GABA in specific areas of the brain. Whether these changes are relevant to the antidepressant action of ECS can only be speculated at present. These studies are relevant for the elucidation of the role of GABAergic mechanisms in the central nervous system (CNS). Current evidence indicates that GABA in the most ubiguitous and important inhibitory neurotransmitter in the mammalian. In fact, GABA appears to be implicated in the pathophysiology of outstanding neurological and psychiatric disorders such as Parkinson's disease and Huntington's chorea. On the other hand, GABAergic circuits are believed to be involved in the expression of many types of behavior, playing the role of inhibitory mechanism directly or indirectly correlated with excitatory ones. Therefore the study of the interrelationship between GABA and dopamine neurotransmission in the CNS should help to classify the biochemical basis of the aforementioned normal and pathological states, thus providing us with a better understanding of human behavior and the background knowledge necessary to develop more effective preventive and therapeutic approaches to a wide range of mental diseases.

We are presently extending these studies by using dopamine agonists in place of electrical stimulation to mimic dopaminergic activity upon GABAergic neuronal populations. Furthermore, techniques designed to overcome the aforementioned difficulties in assessing GABA TR in hippocampus and cerebellum are being developed.

Publications:

Mao, C. C., Cheney, D. L., Marco, E., Revuelta, A. and Costa, E.: Turnover times of gamma-aminobutyric acid and acetylcholine in N. caudatus, N. accumbens, globus pallidus and substantia nigra: Effects of repeated administration of haloperidol. Brain Res. 132: 375-397, 1977.

Marco, E., Mao, C. C., Revuelta, A., Peralta, E. and Costa, E.: Turnover rates of γ-aminobutyric acid in substantia nigra, N. caudatus, globus pallidus and N. accumbens of rats injected with cataleptogenic and non-cataleptogenic antipsychotics. Neuropharmacology, in press.

Green, A. R., Peralta, E., Hong, J. S., Mao, C. C., Atterwill, C. K. and Costa, E.: Alterations in GABA metabolism and met-enkephalin content in rat brain following repeated electroconvulsive shocks. J. Neurochem., in press.

Mao, C. C., Peralta, E., Moroni, E. and Costa, E.: The turnover rate of γ-aminobutyric acid in the substantia nigra following electrical stimulation or lesioning of the strio-nigral pathways. Brain Res., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do **NOT** use this space)

U.S. DEPARTMENT OF
HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 01519-02 SMRP

PERIOD COVERED

October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Selective activation of neuronal systems by cholera enterotoxin

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	L. M. Neckers	Staff Fellow	SMRC	NIMH
Other:	D.-M. Chuang	Chemist	SMRP	NIMH
	R. J. Wyatt	Chief	SMRC	NIMH
	N. H. Neff	Chief	SMRP-B	NIMH

COOPERATING UNITS (if any)

Laboratory of Clinical Psychopharmacology

LAB/BRANCH

Laboratory of Preclinical Pharmacology

SECTION

Molecular Neurobiology and Biochemical Pharmacology

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, St. Elizabeths Hosp., Washington, D.C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.3	0.3	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to evaluate the biochemical changes that occur in neuronal systems after the injection of cholera toxin into selected regions of rat brain. In addition, we are attempting to evaluate the possible mechanism whereby the toxin activates adenylate cyclase.

Project Description:

Cholera toxin is a non-reversible activator of adenylate cyclase. Since adenylate cyclase is thought to be part of the receptor-complex in many neurons, we used cholera toxin to study whether adenylate cyclase was linked to regulatory receptors of the rat septum. In addition, we have attempted to study the biochemical mechanisms that might come into play when adenylate cyclase is activated by the toxin.

Cholera toxin was injected into the septal nucleus of the rat and body weight and adenylate cyclase were studied. The biochemical mechanisms for activation of adenylate cyclase, either whole toxin or its protomers, were studied in vitro.

Cholera toxin, stereotaxically injected into the medial septal nucleus of the rat, led within 24 h to a dramatic decrease in body weight and an increase in septal adenylate cyclase activity. Toxin-treated rats drink one-third the water of vehicle-treated animals while excreting two-and-one half times the urine. Food intake over the 24-hour period was depressed to 13% of control but feces production was normal. The dramatic increase of urinary output suggests that cholera toxin activates a septal adenylate cyclase system which suppresses the release of antidiuretic hormone.

The process of adenylate cyclase activation by toxin appears to take place in three stages: a rapid binding of cholera toxin to ganglioside GM1 on the cell surface, a characteristic lag period, and then a rise of cyclase activity. Cholera toxin consists of a subunit A and a subunit B. The A unit is responsible for activating the cyclase. Nicotinamide adenine dinucleotide (NAD) is required for activation. The A unit hydrolyzes NAD. Diptheria toxin is similar to cholera toxin in that in its active form it consists of two units, a binding unit and a unit that hydrolyzes NAD. With diphtheria toxin, the adenosine diphosphate ribose generated by the hydrolysis of NAD is transferred to an acceptor protein which then modifies protein synthesis within cells. We discovered that the hydrolysis of NAD by cholera toxin results in the ribosylation of the A unit itself. We also found that cholera toxin apparently functions as a adenosine diphosphate ribosyl transferase when glycohormones with amino acid sequences similar to the A unit of toxin or $ACTH_{1-24}$ are present in the incubation medium.

Many psychiatric disorders are associated with abnormal metabolism of the biogenic amines in brain. Adenylate cyclase can be activated by the biogenic amines. Cholera toxin offers the opportunity for understanding the mechanism for activating the cyclases of brain for prolonged periods.

We will continue our studies of the systems that can be activated by the toxin, and of the basic mechanism that underlie the activation of the neurons.

Publications:

Trepel, J. B., Chuang, D.-M. and Neff, N. H.: Transfer of ADP-ribose from NAD to choleragen: A subunit acts as catalyst and acceptor protein. Proc. Natl. Acad. Sci. USA 74: 5440-5442, 1977.

Neckers, L. M., Neff, N. H., Garrison-Gund, C. K. and Wyatt, R. J.: Increased adenylate cyclase activity and rapid weight-loss following intraseptal injection of cholera toxin. Eur. J. Pharmacol., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 01520-02 SMRP

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)
The dynamics of catecholamine metabolism in the CNS

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. W. Commissiong	Visiting Associate	SMRP	NIMH
Other:	S. Gentleman	Pharmacologist	SMRP	NIMH
	N. H. Neff	Chief	SMRP-B	NIMH

COOPERATING UNITS (if any)
None

LAB/BRANCH
Laboratory of Preclinical Pharmacology

SECTION
Biochemical Pharmacology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, St. Elizabeths Hosp., Washington, D.C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.3	1.3	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to identify and study the metabolism of catecholamines in brain and spinal cord and to evaluate the factors that influence amine metabolism. Our present interests are to determine whether dopamine containing neurons are present in the spinal cord and to provide information about their biochemical properties.

PHS-6040
(Rev. 10-76)

Project Description:

Little attention has been given to the possible presence of dopaminergic neurons in the spinal cord because of the lack of sensitive and specific methods for estimating dopamine when it is present in neuronal tissues in low concentrations. Our objective was to use a gas chromatographic-mass fragmentographic (GC-MS) method which is the most specific and sensitive method available to the neurobiologist, to look for dopamine-containing neurons in spinal cord. This dopamine would serve as a transmitter rather than as a precursor for norepinephrine.

Brain and spinal cord were prepared for GC-MS analysis or fluorescent histochemistry. Drugs that modify catecholamine metabolism in brain were administered to determine if they modify catecholamine metabolism in cord. In some studies, brain nuclei were destroyed by electrolytic lesions.

Norepinephrine (NE), dopamine (DM) and 3-methoxy-4-hydroxyphenylacetic acid (HVA) content were measured in different parts of rat spinal cord and cerebellum. In cerebellum, which does not contain dopaminergic neurons, the ratio of NE to DA content was 47, whereas in parts of the spinal cord this ratio varied between 11 and 19. In the cord after desipramine (prevents uptake of catecholamines into noradrenergic nerve endings) plus 6-hydroxydopamine (6-HDA, a specific neurotoxin for catecholinergic neurons, injected intracisternally), there was a significant depletion of DM but not of NE. Conversely, after benztropine (prevents uptake of catecholamines into dopaminergic nerve endings) plus 6-HDA there was a significant depletion of NE but not of DM. Chlorpromazine or clozapine caused a significant increase in spinal cord HVA concentration 1 hr after treatment. Experimental evidence suggested that the increased HVA measured in the cord did not originate in the brain. After electrolytic lesion of the locus coeruleus there was a significant reduction of NE but not of DM. Spinal cord DM and NE were depleted by reserpine in a dose-dependent manner, the threshold dose for DM depletion being less than that for NE depletion. Seven days after cord transection at T_{10} spinal cord DM was significantly reduced in the lumbar region. These results suggest that dopaminergic neurons exist in rat spinal cord independently or noradrenergic neurons and that the DM is likely to be present in the terminals of descending axons.

In the course of our studies of the dopaminergic neurons which project to the spinal cord, we lesioned several brainstem nuclei, including the locus coeruleus. Our consistent observation has been a nearly complete absence of fluorescent terminals from the spinal ventral columns following bilateral lesion of the locus coeruleus. In these same animals, there was a concomitant reduction in the cord of NE but not of DA.

Controversy has surrounded the presence of dopaminergic neurons in the cord, which is based primarily on the lack of specific analytical methods used in the past. We have identified dopaminergic neurons in the cord and have shown that they respond to drugs like the neurons of brain. Abnormal metabolism of dopaminergic neurons in brain has been implicated in several psychiatric and neurologic disorders. Our studies suggest that abnormal DA metabolism could extend to the cord and be involved in much of the abnormal motor movements associated with psychiatric and neurologic disorders as well as with some of the side effect to drugs. The newly identified noradrenergic projection from locus coeruleus to the spinal ventral columns may inhibit the alpha motor neurons during paradoxical sleep, thereby preventing movement and possible injury when EEG recordings show that the brain is aroused with an electrical pattern resembling the awake state.

In the future, we will attempt to identify the physiological function of the spinal cord dopaminergic and noradrenergic neurons.

Publications:

Karoum, F., Neff, N. H. and Wyatt, R. J.: The dynamics of dopamine metabolism in various regions of rat brain. Eur. J. Pharmacol. 44: 311-318, 1977.

Commissiong, J. W., Galli, C. L. and Neff, N. H.: Differentiation of dopaminergic and noradrenergic neurons in rat spinal cord. J. Neurochem., in press, 1978.

Commissiong, J. W., Hellstrom, S. O. and Neff, N. H.: A new projection from locus coeruleus to the spinal ventral columns: Histochemical and biochemical evidence. Brain Res., in press, 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 01521-03 SMRP

PERIOD COVERED
October 1, 1977 to September 30th, 1978

TITLE OF PROJECT (80 characters or less)
Functional role of substance P in brain

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.:	J. S. Hong	Staff Fellow	SMRP-NIMH
Other:	H.-Y.T. Yang	Pharmacologist	SMRP-NIMH
	J.P. Schwartz	Staff Fellow	SMRP-NIMH
	E. Costa	Chief	SMRP-NIMH

COOPERATING UNITS (if any)
None

LAB/BRANCH
Laboratory of Preclinical Pharmacology

SECTION
Molecular Neurobiology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Saint Elizabeths Hosp., Washington, D. C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In order to study the functional role of substance P in substantia nigra, we have studied the interaction between substance P and dopamine neurons. Chronic treatment of rats with haloperidol resulted in a significant decrease of substance P concentration in substantia nigra; in contrast, that of other regions such as striatum and hypothalamus was not affected. This finding suggests that a dopamine neuron may be involved in regulating substance P content in the substantia nigra. A decrease in the substance P concentration occurred only after chronic treatment, and not after acute injection of the drug, indicating that a large pool of substance P precursor may exist. We will use the chick embryo dorsal root ganglion to study whether a precursor(s) is involved in the formation of substance P.

PHS-6040
(Rev. 10-76)

Project Description

It has been suggested that substance P may act as a transmitter or as a modulator in primary sensory neurons of spinal cord. The functional role of Substance P in the brain, however, is not clear. In order to learn more about the interrelationship of substance P with other transmitters, i.e., dopamine, we have studied the effects of antipsychotic drugs, endowed with dopaminergic receptor blocking activity, on the concentration of substance P in various brain regions.

A single injection of a high dose of haloperidol (2 mg/kg) failed to change the Substance P concentration of substantia nigra. However, chronic administration of haloperidol (1 mg/kg/day) for one week caused a 30% reduction of the substance P concentration of substantia nigra. A longer treatment caused a greater decrease in the peptide concentration of substantia nigra (a 40% reduction after two weeks of treatment). In order to understand the mechanism involved in the action of haloperidol on the concentration of substance P in substantia nigra, we have examined the content of this peptide in various brain regions. Rats receiving haloperidol for three weeks (1 mg/kg/day) exhibited a 40% reduction of substance P content in substantia nigra; whereas the substance P content of the hypothalamus and striatum remained unaltered. Hence, the haloperidol action on brain substance P content appears to be circumscribed to the substantia nigra. The effects of pimozide and clozapine on the substance P content of substantia nigra were examined. The concentration of substance P decreased after three weeks of treatment with pimozide (1.5 mg/kg/day i.p.), but failed to change after three weeks of treatment with clozapine (10 mg/kg/day i.p.). It is important to note that unlike pimozide and haloperidol, clozapine elicits little or no extrapyramidal side effects.

This study has demonstrated that there is a significant decrease of nigral substance P content following a protracted treatment with haloperidol. Whether or not this action on substance P content relates to the extrapyramidal side effects of haloperidol still remains to be elucidated. However, this study has suggested that a dopaminergic mechanism participates in the regulation of substance P steady-state of substantia nigra.

Evidence is accumulating that many peptides, protein hormones, and transmitters are synthesized as inactive precursors, and that the conversion of the precursor to the active component is an important regulatory point. We used chick embryo dorsal root ganglia, which contains substance P cell bodies, to look for a precursor to substance P. Incubation of the ganglia in vitro for 20-24 hrs resulted in an increased concentration of substance P which was blocked by cycloheximade. Thus, this system appears suitable for such studies. We plan to isolate substance P mRNA, using the antibody precipitation of the polysomes in order to look for the substance P precursor.

Publications:

Hong, J.S., Yang, H-Y.T. and Costa, E.: Substance P content of substantia nigra after chronic treatment with antischizophrenic drugs. Neuropharmacol., in press

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01523-02 SMRP

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)
Regulation of adenylate cyclase activity by the endogenous protein activator

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. E. Gnegy	Staff Fellow	SMRP	NIMH
Other:	P. Uzunov	Visiting Scientist	SMRP	NIMH
	E. Costa	Chief	SMRP	NIMH
	A. Gallorini	Guest Scientist	SMRP	NIMH

COOPERATING UNITS (if any)
None

LAB/BRANCH
Laboratory of Preclinical Pharmacology

SECTION
Molecular Neurobiology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, St. Elizabeths Hosp., Washington, D.C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.6	1.6	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The participation of an endogenous protein activator in the process of adenylate cyclase supersensitivity to endogenous activators was studied. "Chemical denervation" supersensitivity of striatal dopamine receptors was obtained after discontinuation of chronic treatment with antipsychotics. Animals treated with the cataleptogenic antipsychotics, haloperidol and (+)-butaclamol had an amount of the activator in their striatal membranes larger than control rats and the adenylate cyclase activity was more susceptible to DA stimulation than that of control animals. However, rats treated with the non-cataleptogenic clozapine or (-)-butaclamol which is inactive as an antipsychotic had no change in striatal activator content or in the susceptibility of adenylate cyclase to DA stimulation. It is possible that the adenylate cyclase supersensitivity could be accounted for by the increase in the activator protein in the striatal membranes.

PHS-6040
(Rev. 10-76)

This project has been terminated.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01524-03 SMRP

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)
Modulation of dopamine metabolism in the sympathetic ganglion

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	B. Lutold	Guest Worker	SMRP	NIMH
	F. Karoum	Staff Fellow	SMRC	NIMH
Other:	N. H. Neff	Chief	SMRP-B	NIMH

COOPERATING UNITS (if any)
Laboratory of Clinical Psychopharmacology

LAB/BRANCH
Laboratory of Preclinical Pharmacology

SECTION
Biochemical Pharmacology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, St. Elizabeths Hosp., Washington, D.C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.2	1.2	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Dopamine is present in a special type of cell in sympathetic ganglia. The cells are known as small intensely fluorescent cells (SIF cells) because of their appearance after histochemical staining for catecholamines. These cells modulate information transfer from preganglionic neurons to postganglionic neurons. Our present objective was to evaluate the influence of the preganglionic neurons on the metabolism of dopamine in several sympathetic ganglia of the rat. In addition, we evaluated the possible role of SIF cell dopamine metabolism on the development of hypertension in spontaneously hypertensive rats.

PHS-6040
(Rev. 10-76)

Project Description:

Dopamine, released from special cells in sympathetic ganglia known as SIF cells, may function as a transmitter or modulator for slow inhibitory post-synaptic potentials. Our objective was to evaluate the influence of preganglionic neurons on the metabolism of dopamine in rat ganglia. This was accomplished by contrasting dopamine metabolism in normal and surgically decentralized ganglia. In addition, cholinergic drugs were evaluated for their effects on dopamine metabolism. Biochemical data in the literature suggested that catecholamine metabolism might be abnorma. in the celiac ganglion of spontaneously hypertensive rats (SHR). We therefore correlated the development of hypertension in SHR and in control Wistar-Kyoto rats (WKR) with the metabolism of dopamine in celiac ganglion to detect a possible metabolic deficiency for dopamine.

Dopamine and its metabolites were assayed in the rat superior cervical, middle-inferior cervical and celiac ganglia by mass fragmentography. The relationship between the development of hypertension and dopamine metabolism were studied in the celiac ganglion of SHR and WKR.

In the rat superior cervical, middle-inferior cervical and celiac ganglion 3,4-dihydroxyphenylacetic acid (DOPAC) is the major metabolite of dopamine. The concentration of DOPAC in the ganglia was directly related to the rate of formation of dopamine. Treatment with carbachol increased the concentration of DOPAC in all ganglia. Based on both dose- and time-response studies with carbachol, the celiac ganglion appeared to be the most sensitive of the ganglia studied to carbachol administration. Treatment with atropine completely blocked the response to carbachol suggesting that SIF cell dopamine metabolism is mediated by muscarinic receptors.

Hypertension begins to develop in SHR after 4 to 5 weeks of age and becomes stable after about 10 weeks. In WKR, DOPAC levels are high in ganglion at 4 weeks (400 pmol/mg prot) and decrease by about 45% at 6 weeks where they remain for 20 weeks. In contrast, DOPAC levels in SHR are lowest at 4 weeks (200 pmol/mg prot) increase in parallel to blood pressure and at 20 weeks are comparable to the levels in WKR. Sectioning the greater splanchnic nerves reduces DOPAC levels in both SHR and WKR, demonstrating that DOPAC levels are maintained by cholinergic input to the ganglion. NE and DM levels are comparable in the two groups of rats and nerve sectioning has only minimal effect on levels. Sectioning the nerves, however, delays the development of hypertension in SHR. Our studies suggest that young SHR are not able to modulate the sensitivity of sympathetic neurons to incoming cholinergic input to the celiac ganglion. Lack of modulating capacity may play a critical role in the development of hypertension.

Neuroleptic drugs are assumed to be effective in the treatment of some psychiatric disorders because they alter dopaminergic systems. The rat ganglia may be a simple mammalian system for evaluating the pharmacology of these drugs as well as other drugs. Moreover, our studies suggest that abnormal dopamine metabolism in celiac ganglion might be a critical factor in hypertension.

One of the side effects of treatment with neuroleptic drugs are hypotension. We will evaluate the response of the celiac ganglion, which plays a prime role in blood pressure regulation, to the administration of neuroleptic drugs.

Publications:

Karoum, F., Garrison, C. K., Neff, N. H. and Wyatt, R. J.: Trans-synaptic modulation of dopamine metabolism in the rat superior cervical ganglion. J. Pharmacol. Exp. Ther. 44: 311-318, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01525-02 SMRP

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Regulation of nerve growth factor content of nervous system-derived tissues.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. Schwartz	Senior Staff Fellow	SMRP NIMH
Other:	E. Costa	Chief	SMRP NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Preclinical Pharmacology

SECTION
Molecular Neurobiology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, St. Elizabeths Hosp., Washington, D. C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.8	0.8	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Nerve growth factor (NGF) is synthesized and secreted by two cell lines derived from the nervous system, the rat C6 glioma and the mouse NB2A neuroblastoma. The content of NGF is increased in either cell line by agents which elevate cyclic AMP, β-adrenergic receptor agonists for the glioma cells and prostaglandin E1 or adenosine for the neuroblastoma. The increase of cyclic AMP and concomitant activation of protein kinase does not increase the net synthesis of NGF, but rather appears to activate a peptidase which may form NGF from a pro NGF. Research is being carried out to determine 1) the characteristics of the glioma cell protein kinase(s); 2) the precise mechanism by which protein kinase activates the peptidase and 3) the biochemical characteristics of the precursor NGF.

PHS-6040
(Rev. 10-76)

Project Description:

Two nervous system-derived cell lines, the C6 glioma and NB2 neuroblastoma, synthesize and secrete nerve growth factor. The cells are grown in monolayer culture using standard tissue culture methods. β-NGF has been measured by two different assays: a radioimmunoassay utilizing ^{125}I- and antibody to β-NGF, and a bioassay which measures the outgrowth of neurites of dorsal root ganglia from 9-10 day chick embryos. We now have in addition two assays for measuring the α-subunit or peptidase of the large 7s NGF complex: a radioimmunoassay and an enzymatic assay using an artificial substrate.

The content of β-NGF in both cell lines, as well as the amount released to the medium, can be increased by treating the cells with agents which activate adenylate cyclase. Incubation of the glioma cells with a β-adrenergic receptor agonist causes an elevation of cyclic AMP levels that is maximal in 15 minutes. Cyclic AMP-dependent protein kinase is maximally activated in 30-60 minutes, and then returns to basal activity by 3 hrs. The glioma cells contain both a cyclic AMP-dependent, protein kinase. The cyclic AMP-dependent form has been characterized as a Type II protein kinase on the basis of its elution pattern from a DEAE-cellulose column using a salt gradient.

Characterization of the protein kinase is the first step in determining how the cyclic AMP-activated protein kinase is causing an increase in the β-NGF content of the cells. Experiments utilizing blockers of protein or RNA synthesis, such as cycloheximide and Actinomycin D, have provided evidence that at least the early increase (3-6 hrs) in NGF following the elevation of cyclic AMP is not the result of new protein synthesis. However, several lines of evidence suggest that the cells may contain a precursor or pro-β, as has now been reported for mouse submaxillary gland. Incubation of cell homogenates with the α-peptidase results in more immunoassayable β-NGF than in untreated homogenates. Similarly, homogenates from isoproterenol-treated cells contain more β-NGF than those from control cells following an in vitro incubation. The possibility that the cyclic AMP-dependent protein kinase may be involved in this conversion process has been examined. Incubation of protein kinase directly with purified α-peptidase has no effect on peptidase activity. However, the peptidase is essentially inactive in the 7s NGF complex and incubation of this complex with protein kinase results in activation of α-peptidase activity, apparently mediated by dissociation of the complex into the constituent subunits. Further experiments are underway to demonstrate directly the presence of a pro-β or precursor in the cells

and to provide more direct evidence for the involvement of the protein kinase in the activation process by utilizing a specific protein kinase inhibitor.

It has recently been found that estradiol treatment also increases the NGF content of the C6 glioma cells, with a time course which suggests that new protein synthesis is involved. Furthermore, the NGF content of estradiol-grown cells is increased to a larger extent by isoproterenol treatment than that of control cells. This may thus prove a valuable model for study of the regulation of synthesis of the precursor to NGF.

Publications:

Schwartz, J. P., Chuang, D. M. and Costa, E.: Increase in nerve growth factor content of C6 glioma cells by the activation of a β-adrenergic receptor. Brain Research 137: 369-375, 1977.

Schwartz, J. P. and Costa, E.: Regulation of nerve growth factor content in C6 glioma cells by β-adrenergic receptor stimulation. Naunyn Schmiedeberg's Arch. Pharmacol. 300: 123-129, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 01526-02 SMRP

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Modulation of tyrosine hydroxylase activity in retina

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	P. M. Iuvone	Guest Worker	SMRP	NIMH
Other:	N. H. Neff	Chief	SMRP-B	NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Preclinical Pharmacology

SECTION
Biochemical Pharmacology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, St. Elizabeths Hosp., Washington, D.C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.2	1.2	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to study the physiological mechanisms that are responsible for modulating tyrosine hydroxylase activity in the retina. This enzyme is apparently rate-limiting for catecholamine biosynthesis. Our present interest is to determine whether tyrosine hydroxylase activity of retina is modulated by light.

PHS-6040
(Rev. 10-76)

Project Description:

Tyrosine hydroxylase is rate-limiting for catecholamine biosynthesis. Work from this laboratory has shown that brain tyrosine hydroxylase exists in two forms - a basal and activated state. Activation is manifested by an increase of the affinity of the enzyme for a pteridine cofactor resulting in an increase of enzyme activity in the presence of subsaturating concentrations of cofactor. The assumption is that there is a direct correlation between neuronal firing rates and activation of the enzyme. The amacrine cells of retina contain dopamine. These cells are thought to modulate impulse flow from the photoreceptors to the optic nerve. Our objective was to determine whether tyrosine hydroxylase of retina exists in the basal or activated state and whether environmental lighting alters the kinetic state of the enzyme.

Rats were exposed to 12 hr light-dark cycle or constant light or dark for 4 days and the kinetics of tyrosine hydroxylase were evaluated by standard procedures.

Exposure of rats to light for either 15 min or 96 hr increases retinal tyrosine hydroxylase activity. Concomitant with the increase of enzyme activity is a 4-fold increase of DA formation. The molecular mechanism for the increased enzyme activity for the two lighting situations is apparently different. Short-term exposure to light decreases the Km but not the Vmax of the enzyme for the pteridine cofactor, while 96 hr of exposure to light increases the Vmax for tyrosine but has no significant effect on the Km for tyrosine or cofactor. Enzyme activity of rats exposed to 15 min of light decreases to the level found in 96 h dark-adapted rats within 30 min of darkness, while more than 6 hr of darkness are required after 96 h of light exposure. Activation is intrinsic to the eye as exposing the isolated eyes from rats killed in the dark to a strobe light for 1 min increased enzyme activity. Our studies are consistant with the hypothesis that retinal DA formation is modulated by different molecular mechanisms depending on the duration of exposure to light. Short-term exposure to light activates tyrosine hydroxylase while long-term exposure to light results in the formation of more active molecules of enzyme.

Dopamine transmission may be impaired in schizophrenia. This notion developed because many drugs that are used to treat this disorder modify the biochemistry of dopaminergic neurons of brain. For example, they block dopamine receptors which results in a compensatory increase of neuronal firing rates and activation of tyrosine hydroxylase. The retinal dopaminergic system offers a simple model for evaluating the biochemical processes that are responsible for the physiological activation of tyrosine hydroxylase. Only by understanding normal physiological mechanisms will

it be possible to understand pathophysiology and the mechanism of action of drugs used to treat diseases. Moreover, our studies are providing basic information on the biochemical processing of visual information.

In the future, the mechanism for the activation of the enzyme by light and drugs will be studied.

Publications:

Iuvone, P. M., Galli, C. L., Garrison-Gund, C. K. and Neff, N. H.: Light activated tyrosine hydroxylase and increased dopamine synthesis in retinal amacrine neurons. Science, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01527-02 SMRP

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Studies of endogenous opioids using high pressure liquid chromatography

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	T. Bohan	N.I.G.M.S. Fellow	SMRP NIMH
Other:	J. L. Meek	Pharmacologist	SMRP NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Preclinical Pharmacology

SECTION
Group on High Pressure Liquid Chromatography

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, St. Elizabeths Hosp., Washington, D. C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.8	1.8	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Met enkephalin is an opiate-like compound found in brain and in gut. Since gut survives well in vitro, we chose this tissue to study electrically induced release of met-enkephalin. Met enkephalin was measured by high pressure liquid chromatography coupled with radioimmunoassay (RIA) or with RIA alone. We measured the depletion of met enkephalin from guinea pig ileum as a function of stimulation frequency and duration in normal Krebs and in the presence of protein synthesis inhibitors. Studies are in progress on pre- and post-synaptic control of met-enkephalin release.

Project Description:

In the few years since the discovery of the enkephalins as endogenous opiates remarkable amounts of information have accumulated on their occurence and localization and their pharmacological and electrophysiological effects. In spite of these advances, little progress has been made in the study of the dynamics (turnover) of met enkephalin in neurons. Without such methodology, it has been difficult to assess the afferent connections to the enkephalin neurons and to evaluate these changes in the activity. Such knowledge must be obtained in order to understand the participation of this system in the action of drugs for the treatment of pain or mental disorders.

The objectives of this project are to develop approaches for the study of turnover and release.

The methodology employed includes high pressure liquid chromatography (HPLC), radioimmunoassay and in vitro incubations of guinea pig ileum. HPLC is an invaluable tool in these studies due to its ability to separate closely related peptides. We have used this technique to provide the physical separation needed for confirmation of the values for met enkephalin concentration obtained by radioimmunoassay (see publication 1). For routine measurements, we used RIA because of its greater sensitivity and simplicity. In order to study turnover we are examining whether a tetra-peptide metabolite of met enkephalin, gly-gly-phe-met, can be measured. The turnover experiments are based on an analogy with the catecholamine field, where the concentration of the catecholamine metabolite homovanillic acid (HVA) gives information on the turnover of the parent amine. We have previously shown that the major route of inactivation of the enkephalins is the splitting of the N terminal bond resulting in the peptide gly-gly-phe-met. If this compound is not degraded too rapidly to be detectable, measurement of changes in its content might prove to be an index of the turnover of met enkephalin. In other words, this tetrapeptide might be the "HVA" of the enkephalins. We will attempt to measure the compound by radioimmunoassay. Immunoassay requires an antibody and a radioactive ligand. Dr. Yang in this laboratory is trying to raise the antibody in rabbits. We have produced the radioactive tetrapeptide by chemical synthesis starting with 35S methionine by coupling it to N-protected gly-gly-phe, removing the blocking group, and purifying the resulting compound by HPLC. This radioactive material can now be used to test for antibody production, and then for the assay of the compound in tissues.

Our release studies are performed in the gut since this tissue allows prolonged incubation in vitro, with monitoring of

viability by measuring the electrically induced contraction of the muscle. The tissue (either whole segments of guinea pig ileum or dissected longitudinal muscle strips) is incubated with or without electrical stimulation. Met enkephalin assays were performed both on the tissue and incubation medium.

Major Findings:

1) The electrically induced release of [Met^5]-enkephalin cannot be demonstrated in the medium, probably because of rapid breakdown of the released peptide by tissue enzymes.

2) The depletion of tissue [Met^5]-enkephalin (probably due to release) can be observed after stimulation at 10, but not 0.1 Hz.

3) The addition of cycloheximide to the buffer blocks protein synthesis by an extent greater than 90% for at least 6 hours, but produces only a small effect on electrically induced contractions.

4) Cycloheximide addition fails to lower the enkephalin content of the strips or to increase the depletion due to electrical stimulation. These findings suggest that when the gut is electrically stimulated in vitro synthesis cannot maintain tissue enkephalin levels. It must now be determined whether synthesis can occur in vitro, and if so whether it is ribosomal or via cleavage of a larger precursor.

The significance of this work is that it lays the ground work for a simple model for the study of the enkephalin neuron in both normal and opioid tolerant state. We can examine on the presynaptic side whether putative transmitters cause enkephalin release, and whether these compounds affect electrically induced release. These studies can be easily carried out in tissues tolerant to morphine taken from the same animal, since tolerance can be developed in vitro by incubation of the tissue 24 hr in the presence of morphine.

Publications:

Bohan, T. P. and Meek, J. L.: Met enkephalin: Rapid separation from brain extracts using high pressure liquid chromatography, and quantiation by binding assay. Neurochem. Res. 3: 367-372, 1978.

Meek, J. L. and Bohan, T. P.: Use of high pressure liquid chromatography (HPLC) to study enkephalins. In Costa, E. and Trabucchi, M. (Eds.) Advances in Biochemical Psychopharmacology Vol. 18. New York, Raven Press, 1978, pp. 141-148.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01528-02 SMRP

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Characterization of the neurochemicals involved in the carotid body chemoreceptor mechanisms

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	S. Hellstrom	Visiting Fellow	SMRP NIMH
Other:	E. Costa	Chief,	SMRP NIMH

COOPERATING UNITS (if any)
Section on Biochemical Pharmacology
Hypertensive Endocrine Branch
Heart and Lung Institute

LAB/BRANCH
Laboratory of Preclinical Pharmacology

SECTION
Molecular Neurobiology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, St. Elizabeths Hosp., Washington, D. C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.25	1.25	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

We have characterized the involvement of catecholamines and other putative neurotransmitters in the carotid body chemoreceptor mechanisms, mainly during conditions of hypoxia. Current studies suggest that cholinergic and hormonal control may modulate a physiological role played by dopamine and and norepinephrine in chemoreceptor excitability.

PHS-6040
(Rev. 10-76)

Project Description:

This project has been terminated.

Publication:

Hanbauer, I. and Hellstrom, S.: The regulation of dopamine and noradrenaline content and turnover-rate in the carotid bodies of rats and its modification by sinus and sympathetic nerve denervation and by hypoxia. J. Physiol. (L), 1978 (In press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 01529-02 SMRP

PERIOD COVERED
October 1st, 1977 to September 30th, 1978

TITLE OF PROJECT (80 characters or less)

Possible role of angiotensin as a neurotransmitter in central nervous system.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.	W. Fratta	Visiting Associate	SMRP-NIMH
Other:	H.-Y. Yang	Pharmacologist	SMRP-NIMH
	J. S. Hong	Staff Fellow	SMRP-NIMH
	E. Costa	Chief	SMRP-NIMH

COOPERATING UNITS (if any)
None

LAB/BRANCH
Laboratory of Preclinical Pharmacology

SECTION
Molecular Neurobiology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Saint Elizabeths Hosp., Washington, D. C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.4	0.6	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In order to understand the role of angiotensin in central nervous system angiotensin I converting enzyme which was highly localized in striatum was studied. Our results revealed the neuronal localization of the enzyme in the striatum. The possible neurotransmitter role of angiotensin in the central nervous system will be explored further by studing the regional distribution and regulation of angiotensin in brain.

PHS-6040
(Rev. 10-76)

This project has been discontinued.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 01530-02 SMRP

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Regional distribution and regulation of enkephalin in brain

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. S. Hong	Staff Fellow	SMRP	NIMH
Other:	H.-Y. T. Yang	Pharmacologist	SMRP	NIMH
	W. Fratta	Visiting Associate	SMRP	NIMH
	E. Costa	Chief	SMRP	NIMH

COOPERATING UNITS (if any)

Laboratory of Clinical Psychopharmacology - SMR-IR-MH

LAB/BRANCH
Laboratory of Preclinical Pharmacology

SECTION
Molecular Neurobiology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, St. Elizabeths Hosp., Washington, D.C. 20032

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.8	1.8	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In order to understand the role of met-enkephalin in neurotransmission and the possible involvement of this endogenous peptide in psychiatric disorders, we have studied the effect of some psychotropic drugs on the [met5]-enkephalin content in various brain regions. Chronic treatment with haloperidol, pimozide, chlorpromazine and clozapine, antipsychotics endowed with dopaminergic receptor blocking activity, led to a marked elevation of the enkephalin content in striatum and nucleus accumbens suggesting an interaction between enkephalinergic and dopaminergic neurons in these two regions. Increase of [met5]-enkephalin content in striatum and nucleus accumbens was also observed in the rats chronically treated with lithium. In order to analyze the role of enkephalin in mediating the effect of these drugs, an attempt was made to study the turnover rate and the biosynthesis of met-enkephalin in brain. The turnover rate of met-enkephalin in brain is slow and the incorporation of radioactive amino acids into enkephalins is made difficult by the presence of large pools of precursors. Two endorphin-like peptides with molecular weight larger than met-enkephalin were detected in bovine caudate and they are neither β-lipotropin or α- or β-endorphin. These two peptides are being studied for their role as possible precursors of [met5]-enkephalin.

Project Description:

Enkephalins have been suggested to serve as transmitters or modulators both in central and peripheral nervous systems. Animal behavioral studies implicate that enkephalins may act as transmitter in regulating pleasure, reward or emotion. Recent clinical trials claiming that naloxone and β-endorphin can influence the behavior of mental patients further raise the possibility that enkephalins may play an important role in psychiatric disorders. We then decided to explore an involvement of enkephalins in mental illness by examining the effects of some psychotropic drugs on the concentrations and/or turnover rates of enkephalins in various brain regions.

The concentration of met-enkephalin (ME) or leu-enkephalin (LE) in various brain regions was determined by radioimmunoassay using specific antiserum against ME and LE respectively. Acute injection of haloperidol (2 mg/kg; i.p.), an antipsychotic with specific dopaminergic receptor blocking activity, failed to alter the concentration of enkephalins in brain. Following the daily injection of the drug (1 mg/kg/day; i.p.) for one week, however, selectively elevated the enkephalins concentration in striatum and nucleus accumbens. Longer period of treatment resulted in a greater increase in the concentration of these two peptides; maximal effect (2-fold increase) was obtained after 3 week's injections. Daily injection of haloperidol with the dosage as low as 0.1 mg/kg for 2 weeks, which is close to the average clinical dose, was already effective to elevate the enkephalins concentration. To evaluate the latitude of this finding, we studied the action of other antipsychotics which are also dopaminergic receptors blockers such as, pimozide, chlorpromazine, clozapine on enkephalin level in striatum. Similar to haloperidol, these three drugs are capable of elevating the enkephalin concentration after chronic treatment. Since dopaminergic receptor blocking activity is the common denominator for the antipsychotics tried, this finding suggests that enkephalin concentration is regulated by dopaminergic mechanism.

In consistent with this notion, interruption of dopaminergic transmission either by hemitransection at hypothalamic level or chronic treatment with reserpine also resulted in an increase in enkephalin content in striatum. The selective elevation of enkephalin content in striatum and nucleus accumbens elicited by haloperidol and other antipsychotics suggests that enkephalin may participate in the actions of antipsychotics.

Lithium (Li) has been widely used for treating the manic-depressive illness with an unknown mechanism. We have studied the effects of Li treatment on enkephalin system. Acute injection of Li Cl (5 m eq/kg, i.p.) failed to alter the concentration of enkephalin in brain. Chronic administration of the same dose of LiCl for one week, however, caused a selective elevation of enkephalin content in striatum and nucleus accumbens. Furthermore, the elevation of enkephalin content elicited by Li is independent of the route of administration since feeding the rats with Li_2CO_3 which resulted in a serum Li concentration close to clinical therapeutic effective concentration (1 m eq/l) caused a 60% increase of striatal enkephalin content. This experiment further

suggests the possible involvement of enkephalins in psychiatric disorders.

In an attempt to determine whether the increase of striatal enkephalin content elicited by chronic treatment with haloperidol or Li is due to an increase in synthesis or a decrease in utilization of these peptides, we have studied the turnover of enkephalin in rats chronically treated with haloperidol, Li or saline after the synthesis of peptides was blocked by intraventricular injection of cycloheximide. The rate of decline of enkephalins after cycloheximide was much faster in haloperidol and Li treated rats than the control rats suggesting that the metabolic rates of enkephalins increase after chronic haloperidol or Li. However, since action of cycloheximide is known to be rather non-specific, the afore-mentioned study can only serve as a preliminary evidence.

In order to establish met-enkephalin participation in neurotransmission and in the mediation of psychotropic drug action, it is important to estimate met-enkephalin turnover rate. As the first step, we have studied the in vivo incorporation of radioactive amino acid into met-enkephalin in the brain. In vivo radiolabelling of met-enkephalin was achieved only by infusion of radioactive amino acid over a long period of time (30 min.). Moreover, the formation of radioactive met-enkephalin continues even 2 hrs after the termination of radioactive amino acid infusion. These observations suggested that the in vivo synthesis of met-enkephalin involves the formation of one or more precursors. To test this hypothesis, we have searched for possible precursor(s) in bovine brain. Two endorphin like peptides with molecular weight larger than met-enkephalin were detected in bovine caudate. The study on the chemical nature of these two peptides revealed that they may contain met-enkephalin sequence within their structure but differ from β-lipotropin or α-and β-endorphins. The two met-enkephalin containing larger peptides will be studied for their role as possible precursors of met-enkephalin in striatum of brain. In turn, the effect of psychotropic drugs on the rate of formation of met-enkephalin from the precursor will be studied.

Publications:

Fratta, W., Yang, H.-Y.T., Hong, J. S. and Costa, E.: Stability of met-enkephalin content in brain structure of morphine-dependent or foot shock stressed rats. Nature 268: 452-453, 1977.

Hong, J. S., Yang, H.-Y. T. and Costa, E.: On the location of methionine enkephalin neurons in rat striatum. Neuropharmacology 16: 451-453, 1977.

Hong, J. S., Yang, H.-Y. T., Fratta, W. and Costa, E.: Determination of methionine enkephalin in discrete regions of rat brain. Brain Res. 134: 383-386, 1977.

Yang, H.-Y. T., Hong, J. S., Fratta, W. and Costa, E.: Rat brain enkephalins: Distribution and biosynthesis. In Costa, E. and Trabucchi, M. (Eds.): Advances in Biochemical Psychopharmacology. New York, Raven Press, 1978, pp. 149-159.

Costa, E., Fratta, W., Hong, J. S., Moroni, F. and Yang, H.-Y. T.: Interactions between enkephalinergic and other neuronal systems. In Costa, E. and Trabucchi, M. (Eds.): Advances in Biochemical Psychopharmacology. New York, Raven Press, 1978, pp. 217-226.

Hong, J. S., Yang, H.-Y. T., Fratta, W. and Costa, E.: Rat striatal methionine-enkephalin content after chronic treatment with cataleptogenic and non-cataleptogenic antischizophrenic drugs. J. Pharmacol. Exp. Ther., in press, 1978.

Yang, H.-Y. T., Fratta, W., Hong, J. S., DiGiulio, A. M. and Costa, E.: Detection of two endorphin like peptides in nucleus caudatus. Neuropharmacology, in press, 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 01531-01 SMRP

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Nerve growth factor in human fibroblasts from controls and familial dysautonomia patients.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. P. Schwartz	Senior Staff Fellow	SMRP NIMH
Other:	E. Costa	Chief	SMRP NIMH

COOPERATING UNITS (if any)
Dept. of Human Genetics
Yale University Medical School
New Haven, Connecticut

LAB/BRANCH
Laboratory of Preclinical Pharmacology

SECTION
Molecular Neurobiology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, St. Elizabeths Hospital, Washington, D. C. 20032

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Skin fibroblasts from human controls or from patients with familial dysautonomia (FD) have been grown in tissue culture. β-nerve growth factor has been measured in extracts from both type of cells, using a radioimmunoassay and a bioassay. The FD cells have a 2-3 fold higher content of NGF as measured by RIA than the controls. However, the biological activity of the FD samples is about one-tenth that of the controls when expressed as biological units of activity per ng β-NGF as measured in the RIA.

PHS-6040
(Rev. 10-76)

Project Description:

Familial dysautonomia (Riley-Day Syndrome) is an autosomal recessive disease, in which patients have impaired autonomic and sensory functions. Symptomatically, these patients resemble animals that have been treated with antiserum to nerve growth factor. It has been suggested that there may be an impairment of nerve growth factor associated with the disease, and there are reports that FD patients have elevated serum NGF levels.

We have chosen to use skin fibroblasts from FD patients, and fibroblasts from paired controls, to study this problem further. The fibroblasts are grown in monolayer culture, using early passage cells and standard tissue culture procedures. Nerve growth factor is assayed using a radioimmunoassay which detects β-NGF as well as the β component of 7s NGF, and also a bioassay in which neurite sprouting from 9 day chick embryo dorsal root ganglia is measured.

NGF increases in both control and FD fibroblasts as they go from log to stationary phase growth. When cells of comparable densities are compared, the FD cells have been found to contain 2-3 times more NGF than control cells, as measured by radioimmunoassay. However, assay of the same extracts by bioassay shows that the FD samples are much less active in this assay. When expressed as biological units of NGF per ng β-NGF (as measured in RIA), there is a ten-fold difference in biological activity.

Research is now being carried on to determine what the chemical differences between the control and FD nerve growth factors are. One interesting possibility is that the FD cells are deficient in the γ subunit of 7s NGF, which is a peptidase thought to convert a pro-β-NGF into the active β-NGF: if this were the case, the FD cells may contain a very high level of pro-β-NGF which is measurable by RIA but has little biological activity.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 01532-01 SMRP

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Regulation of catecholamine receptor

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	D. M. Chuang	Chemist	SMRP NIMH
Other:	E. Costa	Chief	SMRP NIMH

COOPERATING UNITS (if any)

None

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Laboratory of Preclinical Pharmacology

SECTION
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INSTITUTE AND LOCATION
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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.2	0.7	0.5

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

We have attempted to study whether adenylate cyclase functions as a regulator of β-receptors in frog erythrocytes. Exposure of frog erythrocytes to high doses of ℓ-isoproterenol results in a marked decrease in receptor affinity for ℓ-isoproterenol, increase of the intracellular content of cAMP and subsequent activation of a cAMP-dependent protein kinase in the cytosol. The activation of protein kinase persists for several hrs after the cAMP concentration has returned back to normal. Protein kinase approaches the control levels about 12 hr after stimulation with ℓ-isoproterenol. The phosphorylation of several membrane proteins is selectively and sequentially increased when the cytosol protein kinase is activated. Ca^{+++} ion inhibits the Vmax for the stimulation of adenyl cyclase by isoproterenal without affecting the Ka. The Ca^{2+} uptake is enhanced during the increase in membrane protein phosphorylation elicited by the protein kinase activation. Ca^{2+} might play a role in desensitization of adrenergic receptors following high doses of ℓ-isoproterenol.

PHS-6040
(Rev. 10-76)

Project Description:

It is well known that erythrocytes from a variety of species contain catecholamine β-receptors which are coupled to adenyl cyclase. Although extensive characterizations of β-receptor and adenyl cyclase in erythrocytes have been reported, their regulation and physiological importance is unclear. We have initiated our investigation by studying the activation of cytosol cAMP dependent protein kinase in frog erythrocytes following the stimulation of β-receptors. In addition, we have attempted to study the mechanism whereby β-receptor stimulation desensitizes adenylate cyclase.

The cytosol of frog erythrocytes contains a cAMP dependent protein kinase which prefers exogenous histone or protamine as the substrate. Chromatography on a DEAE-cellulose column revealed that this enzyme behaves as a type II protein kinase. Exposure of frog erythrocytes to ℓ-isoproterenol results in an instaneous increase in the intracellular concentration of cAMP. In 15 min after stimulation, the increase of cAMP reaches a peak; the cAMP content then declines and returns to the basal value in one hr. This adenylate cyclase stimulation is associated to an activation of cytosol cAMP-dependent protein kinase is maximal in one and half hrs after isoproterenol application, persists for several hrs and approaches normal values in 12 hrs. The Ka of isoproterenol for the increase in cAMP content and the activation of protein kinase is about 0.4 μM; maximal responses are elicited by 20 μM isoproterenol. Intrinsic activities of the agonists of β-receptor to stimulate adenyl cyclase are parallel with their abilities to activate cytosol protein kinase.

The phosphorylation of membrane proteins was then examined in erythrocytes at various times after stimulus application. Isolated erythrocytes were incubated with radioactive inorganic phosphoric acid to label the intracellular ATP and phosphorylation of membrane proteins was analyzed by sodium dodecyl sulfate polyacrglamide gel electrophoresis. Using the technique of $^{32}P/^{33}P$ double labelling, we found that approximately 10 membrane proteins with molecular weight more than 50,000 were phosphorylated and the phosphorylations of several of these proteins were sequentially increased up to 4 hrs after isoproterenol treatment. In 12 hrs, the increase in the membrane protein phosphorylation was no longer detected; at this time the activation of protein kinase in the cytosol was also approaching the control.

We also found that Ca^{++} ion, in micromolar concentrations, added to isolated membrane preparations, inhibited the Vmax of isoproterenol-stimulated adenyl cyclase without affecting the Ka

of isoproterenol for this activation. The basal activity of adenyl cyclase and the Km of ATP for this enzyme were virtually unaffected by this divalent ion. Since the characteristics of frog erythrocytes adenyl cyclase after Ca^{2+} were very similar to those of desensitization induced by incubation of frog erythrocytes with high concentrations of isoproterenol, we have studied whether the uptake of Ca^{++} changes during the induction of desensitization. Isoproterenol was found to have a biphasic effect on the uptake of $^{45}Ca^{++}$ into erythrocytes. Immediately after the exposure to isoproterenol, the influx of Ca^{++} ion was temporarily reduced and 2 hr after stimulation, the rate of Ca^{++} uptake was in fact faster than that of the control. Alprenol, a β-receptor blocker, did not affect the uptake rate of Ca^{++} but totally abolished the effect of isoproterenol on Ca^{++} transport. In 2 hr after exposure, the intracellular concentration of Ca^{++} ion was also significantly higher than that of the control. At this time, desensitization of adenyl cyclase in erythrocytes occurred.

Our results have shed some light on some physiological roles of the β-receptor present in erythrocytes. The stimulation of β-receptor triggers the activation of cAMP dependent protein kinase in the cell; this activation increases the phosphorylation of membrane proteins which enhances the membrane permeability to Ca^{++} ion. The enhanced uptake of Ca^{++} ion could be a mechanism operative in the desensitization of catecholamine receptors in frog erythrocytes.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 01533-01 SMRP

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)
Distribution and biosynthesis of beta-endorphin in brain and pituitary

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.:	W. Fratta	Visiting Associate	SMRP-NIMH
Other:	H.-Y.T. Yang	Pharmacologist	SMRP-NIMH
	E. Costa	Chief	SMRP-NIMH

COOPERATING UNITS (if any)
None

LAB/BRANCH
Laboratory of Preclinical Pharmacology

SECTION
Molecular Neurobiology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, St. Elizabeths Hospital, Washington, D. C. 20032

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1.0	1.0	0

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Beta-endorphin (60-91), and beta-lipotropin are present in various tissues but are mostly concentrated in the pituitary, particularly in the pars intermedia. In brain, beta-endorphin is highly localized in the hypothalamus; however, it cannot be detected in striatum, cortex or cerebellum (0.01 nmole/mg protein). In contrast, the highest concentration of enkephalin is found in the striatum. This dissimilar distribution of enkephalin and beta-endorphin strongly suggests that beta-endorphin and enkephalin are stored in separate neurons and probably the beta-endorphin immunoreactive material of various molecular weights present in the pituitary and hypothalamus suggests that in both tissues, beta-endorphin is derived from beta-lipotropin which, in turn, is formed from a larger molecular weight precursor presumably 31K beta-endorphin.

PHS-6040
(Rev. 10-76)

Project Description:

Beta-endorphin, the c-fragment of beta-lipotropin, possesses an intense binding affinity for the opiate receptors. Pharmacological investigations have shown that beta-endorphin causes analgesia and catalepsy when injected intraventricularly. Clinical reports tend to implicate an involvement of beta-endorphin in the etiology of mental illness; however, this relationship is still rather tenuous. In order to clarify whether this peptide is involved in mental illness, we have initiated a study concerning the location, metabolism and function of beta-endorphin in brain tissue and pituitary.

To measure beta-endorphin, a radioimmunoassay was developed. Regional distribution of enkephalin in brain and pituitary was determined in rats killed by focussed microwave irradiation. Beta-endorphin is highly localized in the pituitary and especially so in the pars intermedia. In brain, beta-endorphin is highly localized in the hypothalamus, is present in midbrain but it cannot be detected in striatum, cortex, cerebellum. In contrast, the concentration of [met^5]-enkephalin is highest in striatum and very low in pituitary. Thereby, these results strongly suggest that beta-endorphin and [met^5]-enkephalin are stored in separate neuronal systems. It is also very unlikely that beta-endorphin is a precursor of [met^5]-enkephalin in striatum. When pituitary and brain extracts were fractionated by Sephadex G-75 column chromatography and the beta-endorphin present in various fractions was measured by radioimmunoassay, three immunoreactive materials were detected. They were beta-endorphin, beta-lipotropin, and a large molecular weight peptide (presumably the 31K beta-endorphin or ACTH). When intraventricular injections of cycloheximide were given to block translocation, the beta-lipotropin and the 31K beta-endorphin content of pituitary were reduced by an extent greater than the beta-endorphin. This suggests that the two former peptides are precursors for the beta-endorphin in normal pituitary.

We are currently studying: 1) The effects of various psychotropic drugs on the metabolism of beta-endorphin; 2) a possible involvement of beta-endorphin in opiate dependence and addiction; and 3) regulation of beta-endorphin formation from its precursors.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 01534-01 SMRP

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Location and role of [met^5] and [leu^5]-enkephalin in neuronal tissue.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	A. M. Di Giulio	Visiting Associate	SMRP NIMH
Other:	H. -Y. T. Yang	Pharmacologist	SMRP NIMH
	E. Majane	Chemist	SMRP NIMH
	E. Costa	Chief	SMRP NIMH

COOPERATING UNITS (if any)

None

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INSTITUTE AND LOCATION
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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The object of this project is to explore the physiological significance of [leu^5]-enkephalin (LE) in neuronal function. Both [met^5]-enkephalin (ME) and (LE) are stored in neurons and are unevenly distributed in brain. Whether these two peptides exist in the same, or different, neurons still remains unclear. In order to study the role of these two peptides we have developed a specific and sensitive method for the LE assay. The method is simple and can be used for measuring leu-enkephalin in crude tissue extract. A study on the distribution of LE and ME in brain regions of various species indicates that a high concentration between the two enkephalins was also observed in drug-treated rats. Chronic treatment of rats with haloperidol or lithium increases the LE and ME content in striatum and N. accumbens but not in other brain areas. ME and LE are also present in sympathetic ganglia, again deafferentation of the ganglia increases both peptides. The results fail to oppose the view that these two enkephalins may be stored in the same neurons.

PHS-6040
(Rev. 10-76)

Project Description:

In 1975, Hughes et al, isolated from brain two pentapeptides, [met^5]-enkephalin (ME) and [leu^5]-enkephalin (LE) differing only by one amino acid at the c-terminus. These two peptides are stored in neurons and have a similar uneven distribution in various neural tissues. However, it is not known whether they are stored in the same or in different neurons. In rats, the ME concentration is much higher than that of LE (5 to 10-fold). Because of this, and the small cross reactivity exhibited by our LE antibody to the ME, it is difficult to measure LE with accuracy. In order to study the physiological significance of LE in enkephalinergic transmission, it is important to develop a sensitive and specific radioimmunoassay for LE. By selectively modifying the methionine structure with CNBr treatment prior to the radioimmunoassay, we were able to devise a sensitive and specific radioimmunoassay for LE. The method is relatively simple and applicable to crude tissue extracts. With this specific assay, we have reexamined the LE content in brain regions of every species (rat, ox and guinea pig) and found that the concentration is much lower than ME in various brain regions of all the species studied. ME and LE were also present in sympathetic ganglia and again ME was more abundant than LE. In every normal tissue analyzed, ME was 5 to 10-fold more abundant than LE, regardless of the basal values. This parallelism was also observed in the brain of rats treated chronically with either psychotomimetics or Li. Haloperidol and lithium elevate ME and LE contents in striatum and N. accumbens by about the same extent. Moreover, ganglia deafferentation of sympathetic axonotomy also changed the LE and ME content, but the changes never altered the ratio of LE to ME concentrations. The physiological significance of LE and ME in enkephalinergic transmission in central and peripheral systems will be studied.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 01535-01 SMRP

PERIOD COVERED

October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Quatitative multiple ion detection mass spectrometry of neuronal peptides

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. Schmid	Visiting Fellow	SMRP	NIMH
Other:	E. Peralta	Guest Worker	SMRP	NIMH
	D. L. Cheney	Chief	SMRP-M	NIMH

COOPERATING UNITS (if any)

None

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Laboratory of Preclinical Pharmacology

SECTION

Molecular Pharmacodynamics

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, St. Elizabeths Hospital, Washington, D. C. 20032

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0.8	0.8	

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Various dipeptides have been converted to their trifluoroacetylhexafluoro-isopropyl and pentaflouropropionyl-hexafluoroisopropyl derivatives and analyzed under gas chromatographic conditions. The derivatives show good separation properties and can be separated on nonpolar or slightly polar columns. Dipeptidyl aminopeptidase I, which specifically splits polypeptides into dipeptides, has been prepared from beef spleen preparatory to quantitative multiple ion detection mass spectrometry of neuropeptides.

PHS-6040
(Rev. 10-76)

Project Description:

Our objective is to develop a specific and sensitive gas chromatographic-mass fragmentographic method to determine the concentrations of peptides in neuronal tissue.

Various peptides have recently received increased attention as putative neurotransmitter substances, however, available chemical or immunohistochemical methods fail to infer the necessary specificity. To study their role in the CNS the neuropeptide and their precursors concentration in various brain regions under varying experimental conditions must be evaluated.

Peptides may be specifically split into dipeptides by the action of a dipeptidase, dipeptidyl aminopeptidase I as described by Krutzsch and Pisano (Meth. Enzyme 47: 391-404, 1977). The enzyme was isolated from beef spleen by the method of Krutzsch and Pisano (Meth. Enzyme. 47: 391-404, 1977) and McDonald et al. (Meth. Enzyme 25: 272-282, 1972). The beef spleen tissue was differentially precipitated by ammonium sulfate fractionation. Further purification of the enzyme precipitate was done by dialysis, following incubation and a chromatography over a Biogel A column (0.5 m). The specific activity of the preparation was evaluated using the artificial substrate Gly-Phe-Naphtylamine.

The gas chromatographic properties of the various dipeptides were determined. Dipeptides were reacted to yield their trifluoroacetyl-hexafluoroisopropyl and pentafluoropropionyl-hexafluoroisopropyl derivatives. They were then analyzed in the gas chromatograph under various conditions. Both derivatives showed good gas chromatographic properties and most of the dipeptides can be separated on nonpolar or slightly polar columns. Mass spectra of these compounds were run to ascertain their molecular composition and identity.

Since many peptides have been implicated as neuromodulators and neurotransmitters, methods to quantitate concentrations in various pharmacological conditions are absolutely necessary.

These methods will be further worked out for routine laboratory determination of enkephalins in different brain areas and under various pharmacological conditions. Future work includes measurement of various peptides quantitatively using multiple ion detection mass spectrometry.

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